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Vol. II

TRANSCRIPT OF RECORD

(Pages 369 to 840)

Supreme Court of the United States

OCTOBER TERM, 1947

No. 280

FUNK BROTHERS SEED COMPANY, PETITIONER,

vs.

KALO INOCULANT COMPANY

ON WRIT OF CERTIORARI TO THE UNITED STATES CIRCUIT COURT OF APPEALS FOR THE SEVENTH CIRCUIT

PETITION FOR CERTIORARI FILED AUGUST 19, 1947.

CERTIORARI GRANTED OCTOBER 13, 1947.

IN THE

Supreme Court of the United States

OCTOBER TERM, 1946.

No.

FUNK BROTHERS SEED COMPANY,

Petitioner,

vs.

KALO INOCULANT COMPANY,

Respondent.

ON WRIT OF CERTIORARI TO THE UNITED STATES CIRCUIT COURT OF APPEALS FOR THE SEVENTH CIRCUIT.

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IN THE

United States Circuit Court of Appeals

FOR THE SEVENTH CIRCUIT

KALO INOCULANT COMPANY,
Plaintiff-Appellant,

No. 9040 vs.

FUNK BROTHERS SEED COMPANY,
Defendant-Appellee.

KALO INOCULANT COMPANY,
Plaintiff-Appellee,

No. 9041 vs.

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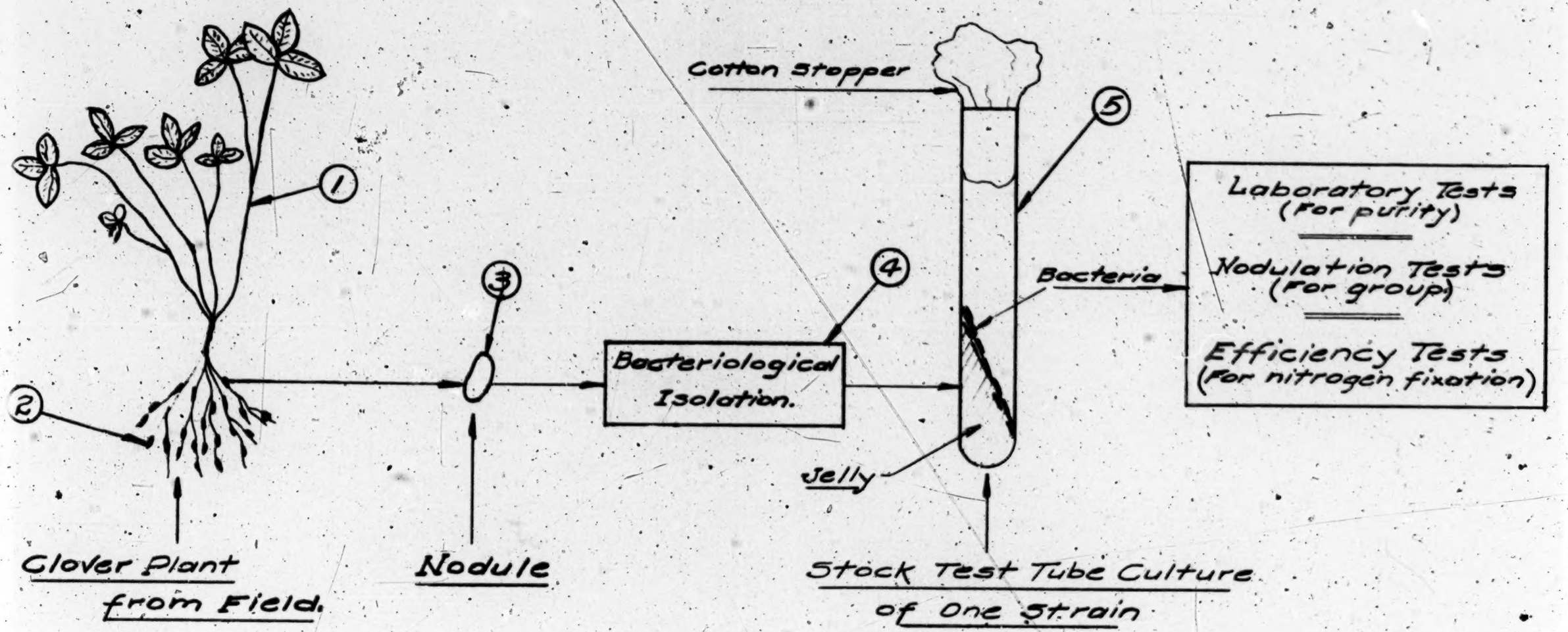
LEGUME-AID
ALFALFA & CLOVER COMBINATION
LEGUME-AID
PLFS. EX. 1 IDEN.
LEGUME-AID
IS USED TO
INCREASE YIELDS
IMPROVE QUALITY
AND BUILD UP
SOIL FERTILITY
IMPORTANT
This carton contains a mixture of two different kinds, or groups, of nodule bacteria.
(1) The Alfalfa Group
For Alfalfa, Sweet Clover, Hubam, Southern Bur Clover, and Black Medic.
(2) The Clover Group
For Red, Alsike, White, Mammoth, Ladino, Hop, and Crimson Clover.
Particularly recommended for inoculating mixtures of Alfalfa and Clover seed, or EACH separately.
INOCULANT
ANOTHER STEP AHEAD
DIRECTIONS
GUARANTEE
Inoculate
ALL LEGUME SEED WITH LEGUME-AID EVERY YEAR
PRODUCED, TESTED AND GUARANTEED BY AGRICULTURAL LABORATORIES INC.
CARTON SIZE NO. 1
369-370

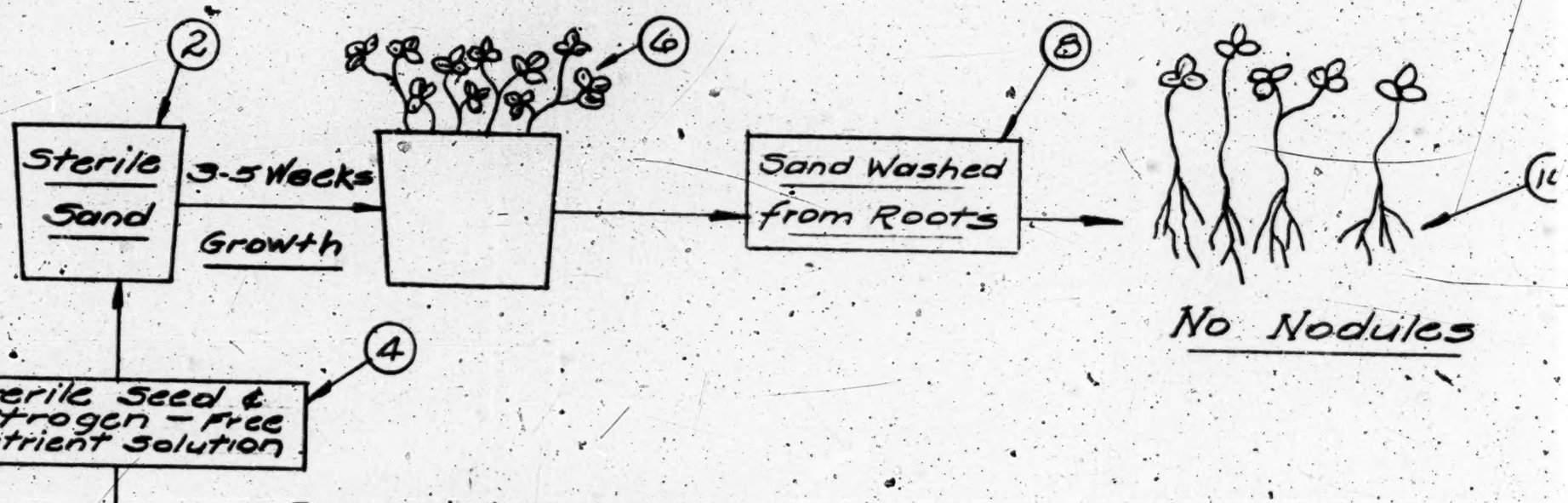
Source of Stock Strains



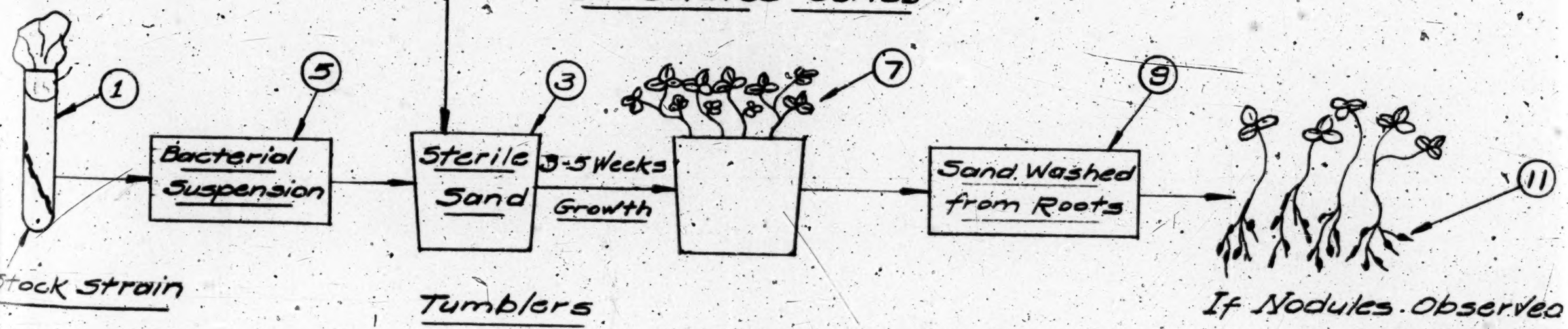
PLFS. EX. 3 IDEN.

Nodulation Test

Uninoculated Control Series

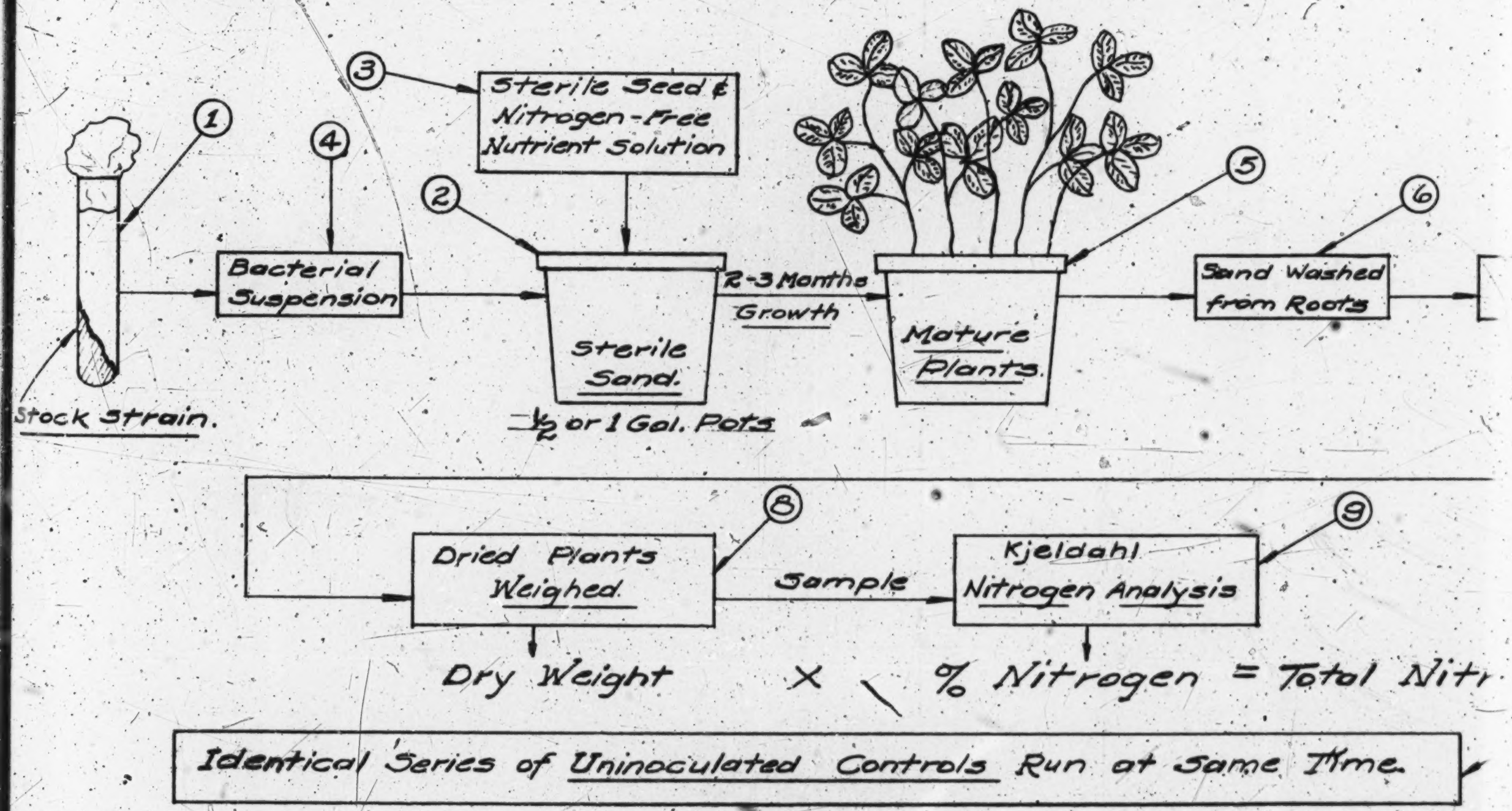


Inoculated Series



PLFS. EX. 4 IDEN.

"Efficiency" Test for Nitrogen Fixation



PLFS. EX. 5 IDEN.

trogen Fixation

5

6

7

ith
th

Mature Plants

Sand Washed from Roots

Plants Dried

9

mple

Kjeldahl Nitrogen Analysis

% Nitrogen = Total Nitrogen

10

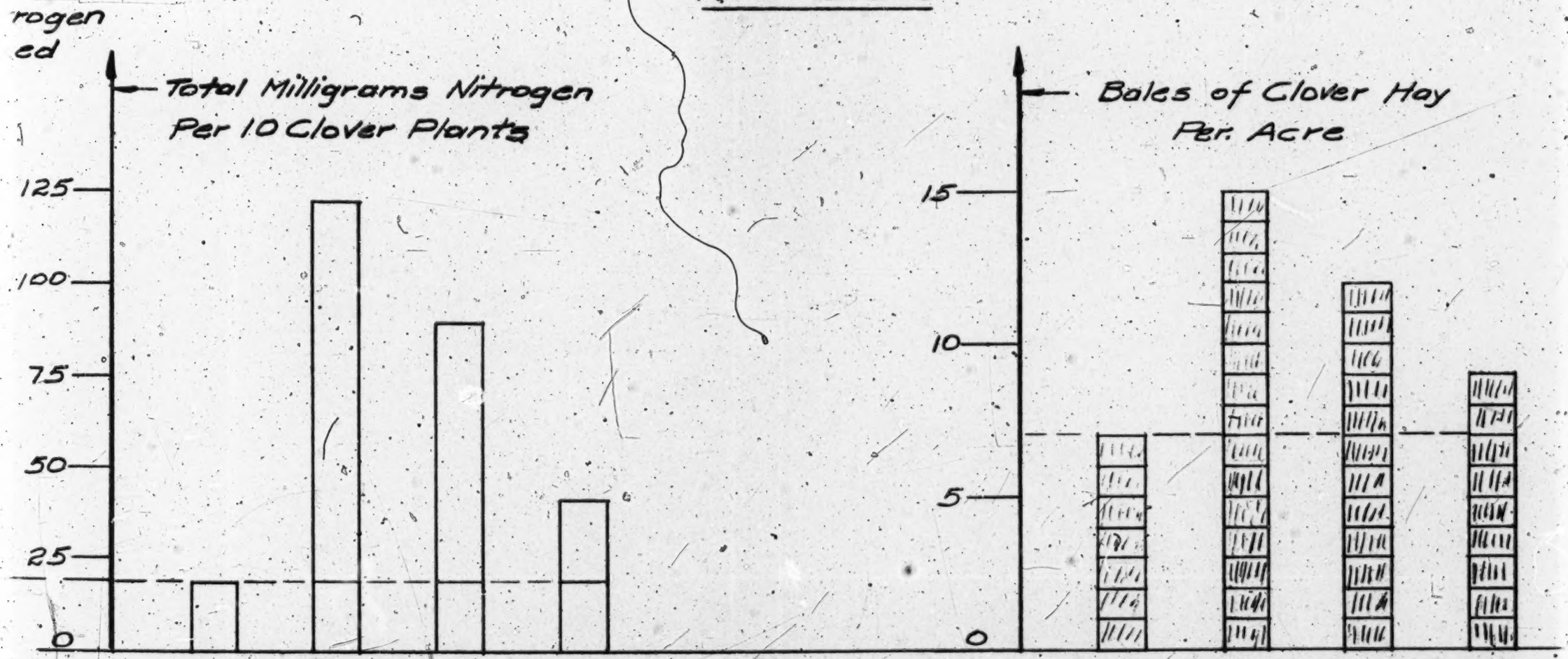
Controls Run at Same Time

n Inoculated - Total Nitrogen Control

PLFS. EX. 5 IDEN.

Importance of "Efficiency" Tests

Red Clover

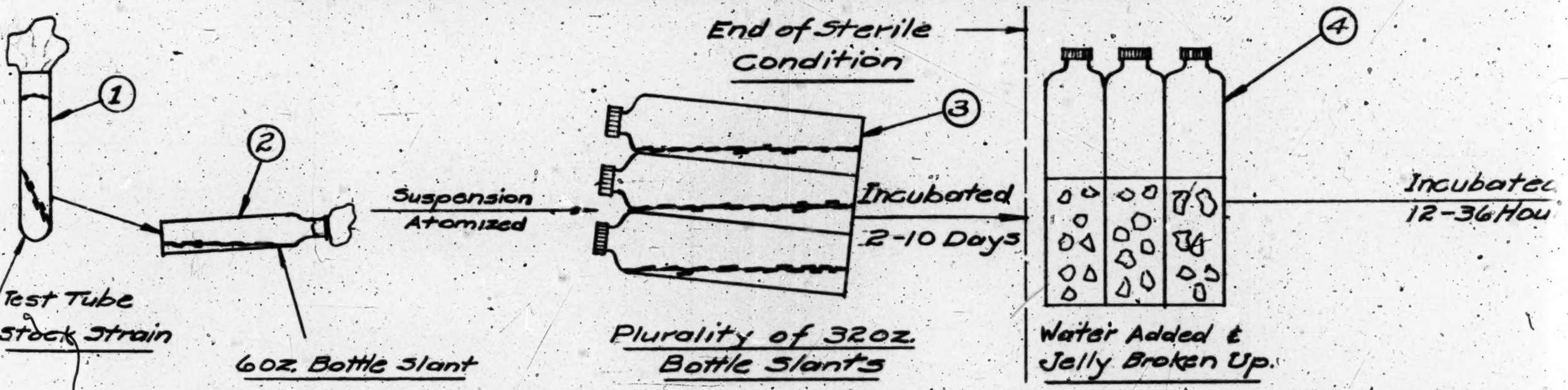


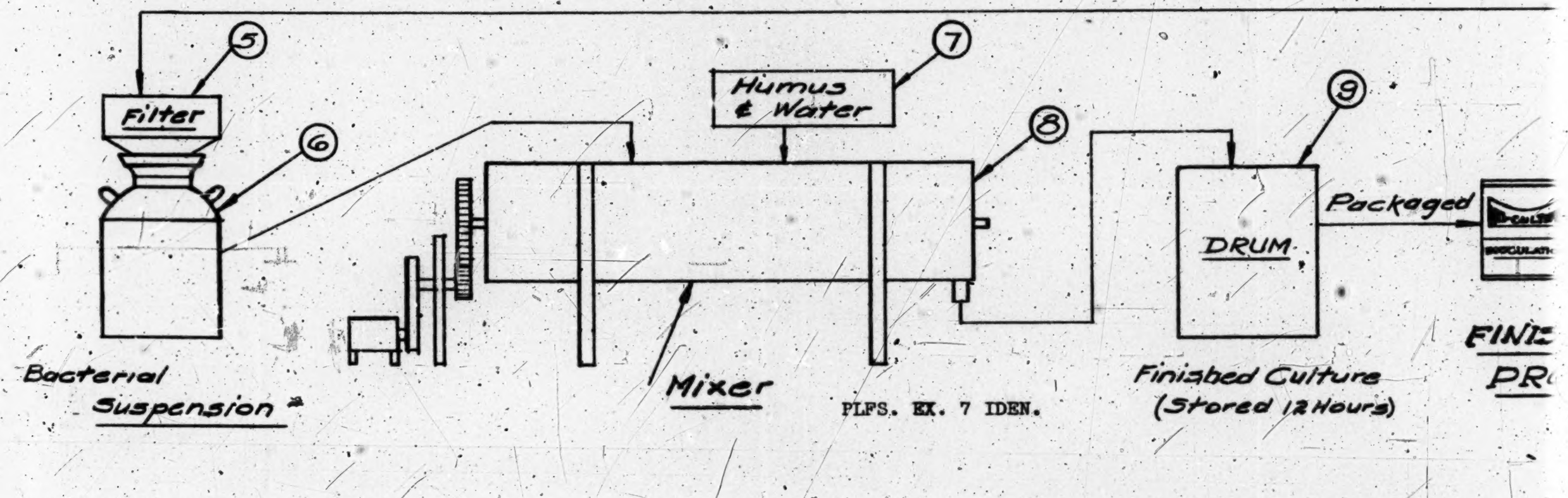
Greenhouse Efficiency Tests

Field Results

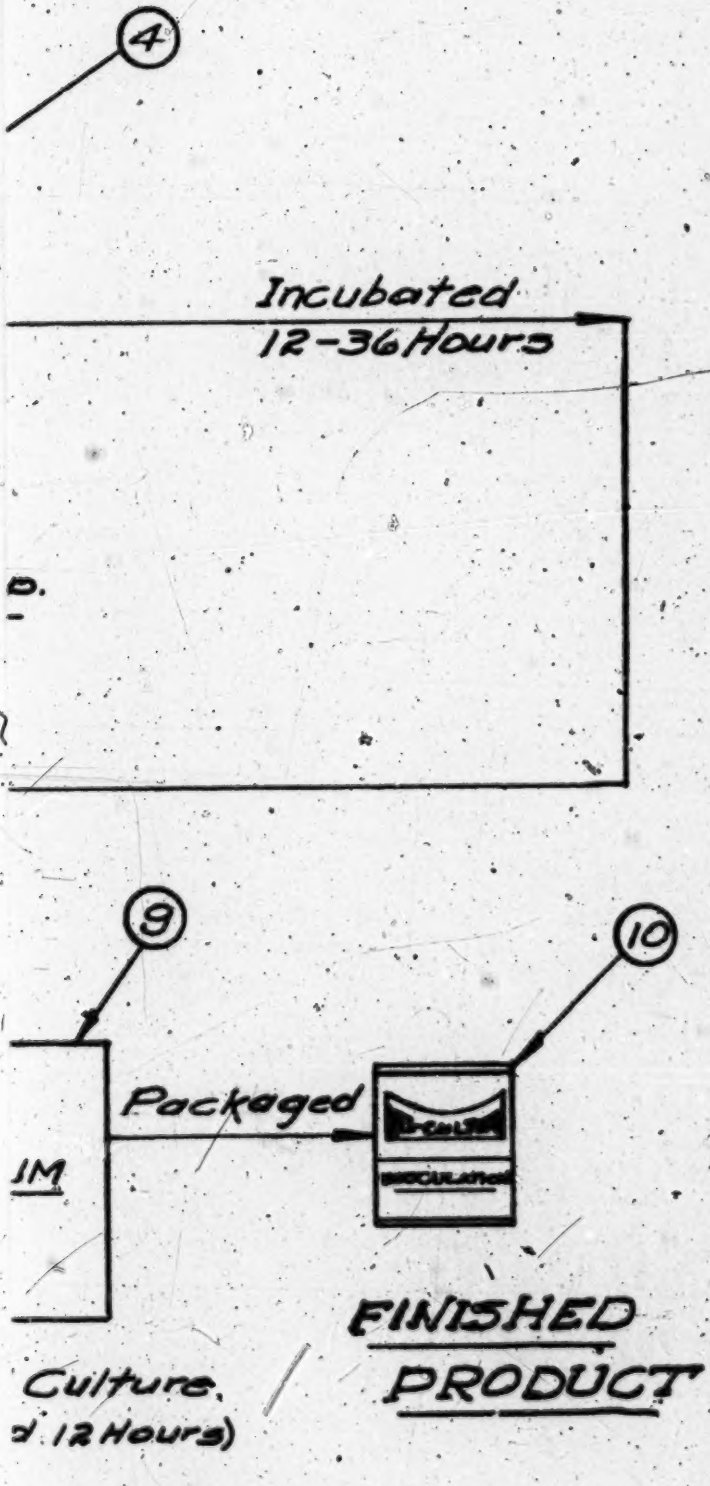
PLFS. EX. 6 IDEN.

Preparation of Humus Type Culture.





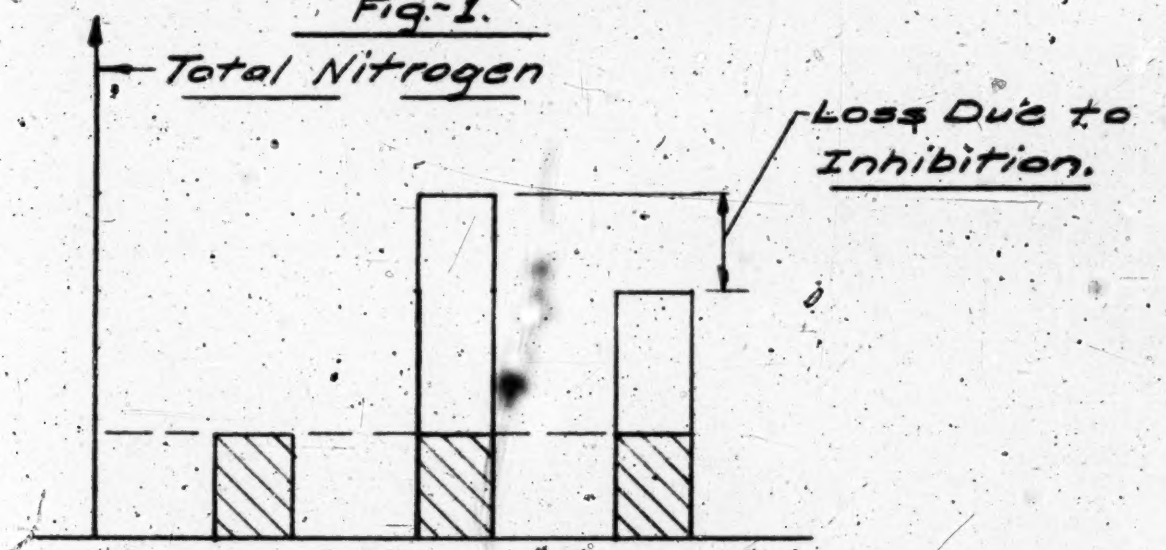
PLFS. EX. 7 IDEN.


4
Incubated
12-36 Hours
D.
9
10
Packaged
IM
FINISHED
PRODUCT
Culture.
d 12 Hours)

Effect of Inhibition in Mixed Culture.

Inhibitive Strain of Bacteria.

Fig.-1.



Non-Inhibitive Strain of Bacteria.

Fig.-2.

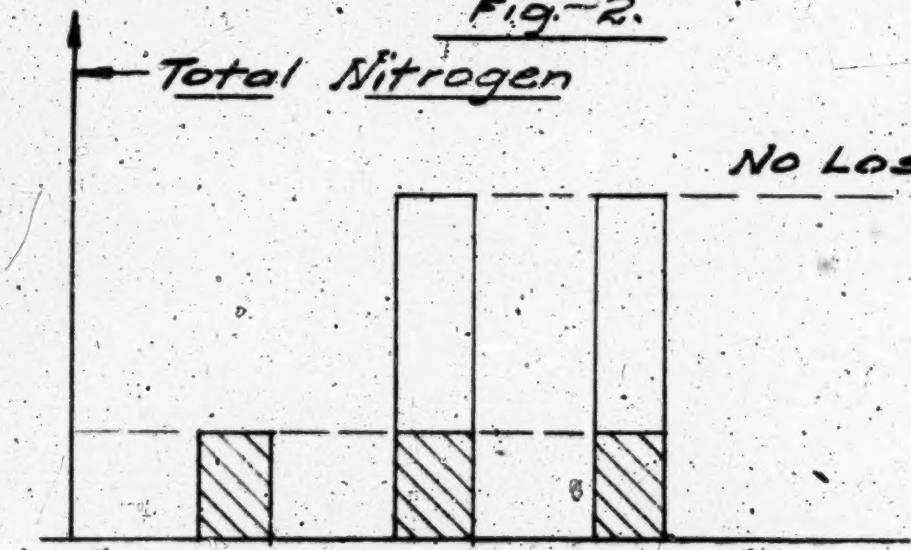


Fig.-3.

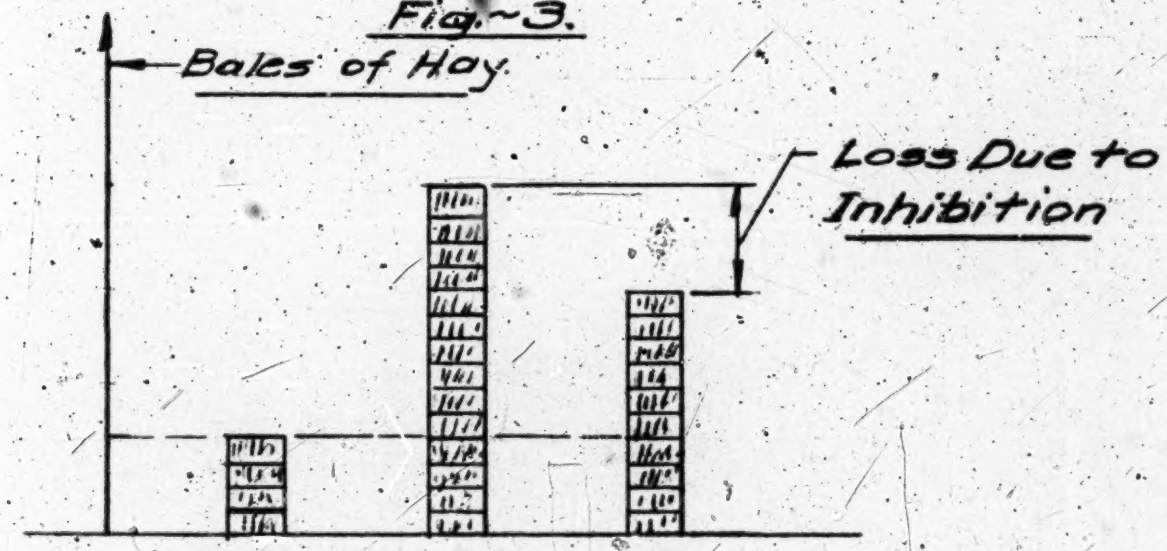
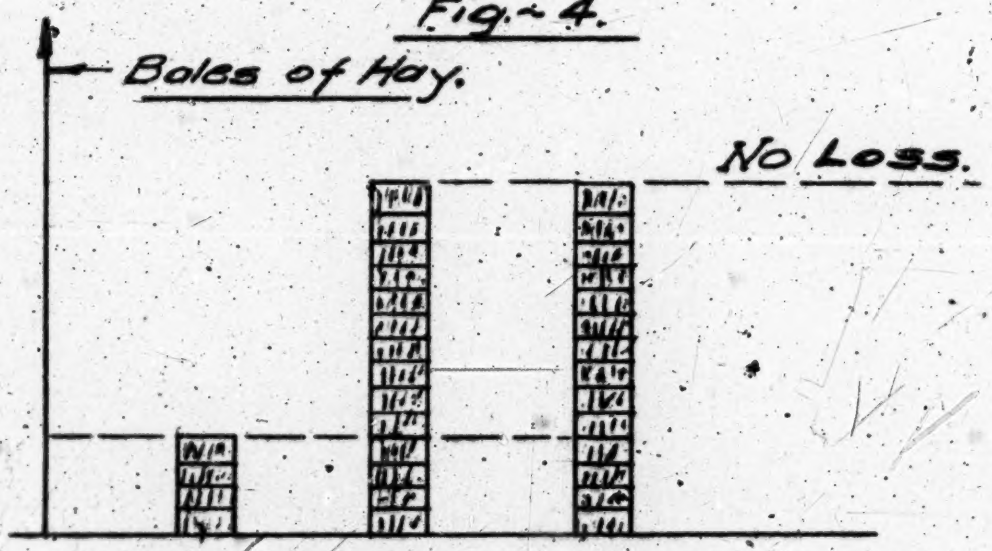


Fig.-4.



PLFS. EX. 10 IDEN.

PLAINTIFF'S EXHIBIT 11.

832 IN THE

DISTRICT COURT OF THE UNITED STATES

SOUTHERN DISTRICT OF ILLINOIS

SOUTHERN DIVISION

KALO INOCULANT COMPANY, a corporation, Plaintiff, vs. FUNK BROTHERS SEED COMPANY, a corporation, Defendant.	Civil Action No. Q-62 Patent Infringement Suit Bond Patent No. 2,200,532

STIPULATED TRANSLATION.

833 IT IS STIPULATED AND AGREED by and between the parties hereto, the Honorable Court consenting, subject to correction should error appear, that the following is a true and correct translation from the German of an article published in *Praktische Blätter für Pflanzenbau und Pflanzenschutz*, Vol. XIV, No. 7 (1936-1937):

On Mutual Retardation and Favoring Between Nodule Bacteria

By Max Kronberger; Bavarian Agricultural Institute for Plant Culture and Plant Protection, Munich, Germany.

The important role which is played by the nodule bacteria in the nitrogen nourishment of the leguminous plants does not require any further proof. Of particular interest, however is the fact that between the individual species of nodule bacteria there subsists mutual injurious and favoring influences which are closely connected with the nitrogen economy and hence also with the growth of the plants. Our previous knowledge on this subject is principally based on the older researches of Gerlach and Vogel and Heinze and L. Hiltner. Gerlach and Vogel (1) were the first to show that the nodule development of inoculated lupines, and, in connection therewith, also the yield of green mass, was only medium when lupine, and

the best when serradella, had been sown as the preceding crop. If, conversely, the inoculated lupines were cultivated after soya beans, then the effect of the inoculation was found to be almost zero. Heinze (2) has established the fact that lupines following after peas remained
free of nodules while after serradella they exhibited
834 a copious covering of nodules and gave twice as much
nitrogen as those cultivated after peas. In the work of L. Hiltner (3) there is found the valuable datum that the intolerability between serradella and red clover is also at least partly due to unfavorable (antagonistic) mutual relations between the nodule bacteria of serradella and red clay. He sums up the results of his research as follows:

". . . It is thus clearly seen that the red-clover bacteria exert an influence on the development of serradella which is even injurious, that is, as was established by microscopic examination, because while these bacteria are attracted by the serradella roots and come to cover them densely they do not penetrate into them but only to a certain extent block the way of serradella bacteria which may come afterward or may be present in small numbers in the ground, in that, as it seems, they neutralize the attracting substance of the roots.".

The present research also constitutes a contribution to this important finding. Through this research it has been sought to ascertain, on the one hand, the action of the nodule bacteria of red clover under the influence of other varieties of nodule bacteria of legumes on red clover, and, on the other hand, the action of the latter types of bacteria under the influence of nodule bacteria of red clover on the particular legumes.

It was our intention only to deal with those types which can be regarded, with respect to their biological behavior toward symbiotic nitrogen-fixing, as particularly characteristic, and which are characteristic of those legumes following which red clover frequently turns out to be a
failure (4). For these reasons we selected the nodule
835 bacteria of the horse bean, pea, vetch, alfalfa, yellow
lupine and serradella.

The arrangements for obtaining the inoculating cultures necessary for the carrying out of the experiments were carried out on June 5, 1935, in such a way that each of the fresh cultures of the nodules bacteria to be tested

(including that of red clover) was brought in the form of a strip onto the sterile nutrient-agar plate, both by itself and at the same time with the particular breed of nodule bacterium concerned, in the form of parallel lines, as uniformly as possible. The composition of the nutrient medium was the same as that employed by the Agricultural Institute for the production of legume inoculating material. The inoculating cultures, in the form of three applications, were left standing for 21 days at room temperature before being used in the experiments. During this period there was not observed any retardation in growth in any case. Both the pure cultures and the mixed cultures exhibited a good growth. The experimental plan can be seen from the recapitulation.

As the experimental vessels there served clay pots, each of which was filled with 2500 grams of Hohenbocka quartz sand and 1000 grams of humus, lime-bearing, unfertilized earth. The fertilization of each of the pots consisted of 2 grams of 40% potash salt and 4 grams of superphosphate. After being filled, the vessels were covered with a germ-free wadding, and kept in the autoclave for 25 minutes at an absolute pressure of 1 atm. superatmospheric. The purpose of the sterilization was the killing of any nodule bacteria that might be present in the soil material employed. The preparation of the seed to be sown
836 consisted in each case of a cauterization with 1% sublimate solution. The duration of the cauterization amounted to 15 minutes. The sowing occurred on June 26th with the employment in each case of 4 times 25 grains per vessel for the red clover, serradella and alfalfa, and 3 times 2 grains with the peas, vetch, horse bean and yellow lupine. The inoculation was carried out immediately after the swelling of the seed in such a way that in each case there was brought into the ground 30 cc of the pure or mixed culture concerned mixed with 150 cc of sterile tap water and with 0.5 gram of thereto added milk powder. In doing so the germinating plant, which has originally been covered by the wadding, was only exposed far enough to allow the stalk to come out. Watering occurred in the course of vegetation under the employment of distilled water. Of the rest of the vegetation notes the following may be mentioned:

Stand on August 6th

1. Red Clover.

The best stand was, as was to be expected, exhibited by the plants which had been inoculated with their own variety of bacterium. The effect of this inoculation as compared with the other series is expressed in the darker leaf color as well as in the higher growth. Under the addition of nodule bacteria of horse bean as well as of vetch to the nodule bacteria of red clover there is observed a good to very good growth. Good, although somewhat weaker, is the action of the red clover alfalfa inoculating-material. The color of the plants may be described as the normal green. Less pronounced is the effect of the mixed culture of nodule bacteria of red clover plus peas. In both cases the growth of the clover is
837 somewhat retarded and its leaves are considerably lighter in color. Obviously more difficult is the intake of nitrogen of the plants inoculated with the nodule bacteria of red clover and serradella. They exhibit in general nearly the same behavior as "uninoculated" plants, are of a light-green leaf color and a low growth.

2. Horse Beans

The best and highest are the horse beans which have been inoculated with their own variety of bacteria. They excel in a green-dark green leaf color. While the addition of red-clover nodule-bacteria to their own variety of culture exhibits a somewhat smaller nitrogen action, its superiority to the uninoculated series is nevertheless clearly expressed in the somewhat darker green of the leaves.

3. Peas

Also here the inoculation with the peas' own culture is the most favorable. The leaf color of the plants owing to the nitrogen supply may be described as dark green. Not very favorable to the growth and the stand of the peas is the addition of red-clover nodule-bacteria to the peas' own culture. Nevertheless the total impression is better than that of the uninoculated series.

4. Vetch

A certain worsening of the inoculating action is un-

doubtedly present in the case of the mixed culture consisting of nodule bacteria of red clover and vetch. The stand of the plants is not as good as with their own inoculating culture, and they are considerably lower in growth. The uninoculated vetches are particularly distinguished by their light-green leaf-color and their small increase in growth.

838 *5. Alfalfa*

The inoculation of alfalfa with its own variety of culture is able to supply its nitrogen requirements. The plants are of an outstandingly fresh-green color. Less favorable is the action of inoculation with the mixed culture of nodule bacteria of red clover and alfalfa. Light-green leaves and low growth characterize the uninoculated plants.

6. Yellow Lupine

The development of the lupines treated with the red clover-lupine inoculating-material is definitely better than that of the uninoculated plants, even though no sharply-defined difference has as yet appeared. Conversely, the inoculation with bacteria of its own kind exhibits a perceptible result, which can be recognized by the lively green of the leaves as well as by the great increase in growth.

7. Serradella

As with lupine so also with this species of legume a considerable favoring of growth is only present in the case of inoculation with its own form of bacteria. Also the color of the leaves is already green-darkgreen. Less favorable, however, is the mixed culture of nodule bacteria of red clover plus serradella. The most unfavorable impression is that of the uninoculated series.

August 27th

The superiority of the cultures of their own kind is more sharply pronounced in all of the series. The plants exhibit the most vigorous development, dark-green leaf-colors, and copious new growth. While the leguines inoculated with the mixed cultures clearly exhibit a nodule action, there are observed, on the whole, within the indi-

vidual comparative series, differences similar to those of August 6th.

839 *September 8th*

A considerable favoring of lupine is now also present with the mixed culture, although not to the same extent as with its own culture.

The harvesting in such case was now carried out on a date at which the development of the green mass had proceeded farthest. On September 2nd there were harvested horse bean, peas, vetch and serradella. The first cutting of the red clover and of the alfalfa occurred on October 7th and the second on November 16th. On the same day the lupine was also harvested.

The plants gave, in a mean of 3 comparative vessels, the following values in dry weight (see recapitulation on page 207 (of original German text)).

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	grams						greater (plus) or less (minus) yield compared with	
	first cutting			second cutting		total	uninoculated	plant's own culture
							%	%
serradella								
uninoculated	3.80	plus-or-minus	0.12	plus-or-minus	...		plus	....
inoculated with serradella nodule bacteria	8.67	"	0.26	.....	...		" 128.16	....
ditto plus red clover nodule bacteria	6.67	"	0.66	.....	...		" 75.53	—23.07
horse bean								
uninoculated	7.83	"	1.26	.....	...		....	....
inoculated with horse-bean nodule-bacteria	20.33	"	1.86	.....	...		" 159.64	....
ditto plus red clover nodule-bacteria	19.00	"	0.40	.....	...		" 142.66	— 6.54
peas								
uninoculated	6.83	"	0.33	.....	...		....	....
inoculated with peas nodule bacteria	13.66	"	0.53	.....	...		" 100.00	....
ditto plus red clover nodule bacteria	10.17	"	0.53	.....	...		" 48.90	—25.55
vetch								
uninoculated	5.27	"	0.26	.....	...		....	....
inoculated with vetch nodule bacteria	7.50	"	0.20	.....	...		" 45.07	....
ditto plus red clover nodule bacteria	6.00	"	0.20	.....	...		" 18.6[illegible]	—20.00

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yellow lupine								
uninoculated	9.00	plus-or-minus	0.40	.....	...	plus	....	
inoculated with lupine nodule bacteria	24.17	"	0.23	.....	...	" 168.55	....	
ditto plus red clover nodule bacteria	21.33	"	1.26	.....	...	" 137.00	—11.75	

Plaintiff's Exhibit 11

843 As can be seen from the values listed in the accompanying recapitulation all of the plants inoculated with their own bacterium culture exhibit a considerable increase in yield. But neither has the mixed culture in any case remained without action as compared with the "uninoculated" plants.

Examining now to what extent the inoculation effect of the red-clover nodule-bacteria is influenced by the nodule bacteria of the vetch, it is seen that no considerable decrease in the clover yield has resulted therefrom. The decrease in the harvest only amounts to 4.97%. A somewhat greater decrease in the harvest, that is, only 5.40% was brought about through the addition of horse-bean nodule-bacteria. However, also here the results are only slightly worse than those of the plant's own culture. While neither of these two differences is, as already mentioned, particularly appreciable, they must nevertheless be taken into account. Already more clearly expressed is the decrease in harvest with the red clover-alfalfa inoculation-culture, in which the deficit brought about through the addition of the alfalfa nodule bacteria has risen to 9.25%. A greater decrease in the harvest value is due to the addition of the peas nodule bacteria, in that there is here found a deficit of 16.98%. A still more unfavorable effect occurred through the mutual influence between the red-clover and the lupine nodule-bacteria, which, with a decreased yield of 19.55%, occupies the second-last place. Under the influence of the nodule bacteria of serradella, finally, the nitrogen-fixing action of the red-clover nodule-bacteria was by far the least, the loss in harvest amounting to 33.91%.

844 Moreover, in the attempt to establish to what extent the inoculation action of foreign nodule-bacteria cultures under the influence of the red-clover nodule-bacteria is expressed on the harvest of the various legumes, there are also found greater or less increments. The least reactive were the nodule bacteria of the horse bean. Here the decrease in harvest as compared with that of the plant's own type of culture amounts to 6.54%. Next come the nodule bacteria of yellow lupine. With it the decrease in harvested material has already risen to 11.75%. This is true to even a greater degree of the nodule bacteria of vetch, whose nitrogen-accumulating ability has already suffered a loss of 20%. In comparing with each other

the results determined for alfalfa there is noted a deficit of as much as 21.54%. Still worse results were brought about through the addition of red-clover nodule-bacteria to the nodule bacteria of serradella, the yield of the latter dropping 23.07% therethrough. By far the most pronounced, finally, was the retarding action of the foreign culture with peas, in which the loss in harvest brought about therethrough amounts to 25.55%.

In considering the cause of the depression in the nitrogen-fixing ability of the native culture through a foreign culture the first thing that comes to mind is a greater or less withdrawal of nutrient by the accompanying organism. This view is not, however, in the least indicated by the result of our experiments in this direction. It was found, that is, that, upon the reinoculation of red clover or any of the other legumes which had already developed normally for 3 weeks, the growth of the red-clover nodule-bacteria continued to proceed well, even if not so well as on the control bed of the same age.
845 It must, however, be noted in this connection that in the nutrient soil which had previously served for the culture of serradella nodule bacteria, the red clover nodule bacteria started considerably more slowly. The reinoculation of the nutrient soil never occurred in any case until after the removal of the bacteria layer followed by sterilization thereof. Accordingly, it may here be assumed that it is obviously less the nutrient withdrawal than the effect of the by-products of the metabolism of the added type of nodule bacteria which has exerted the decisive influence on the nitrogen-fixing ability of each of the particular native culture concerned. Finally, the circumstance that the nitrogen-fixing ability is influenced through the presence of the one and the other type more and less favorably respectively, would seem definitely to be due more to the difference in the nature than to the difference in the quantity of the products of metabolism of the various types of nodule bacteria tested.

The present work thus again shows that the nodule bacteria of various kinds of legumes do not remain uninfluenced. Particularly from the results determined for red clover there can be seen the greater or less unfavorable influence occurring therethrough on the nitrogen-accumulating ability of red-clover nodule-bacteria and hence also on the yield of clover itself. However, the results

	grams							greater (plus) or less (minus) yield compared with	
	first cutting			second cutting			total	uninoculated	plant's own culture
								%	%
red clover									
uninoculated	13.33	plus-or-minus	0.53	2.27	plus-or-minus	0.18	15.60	....	....
inoculated with red-clover nodule-bacteria	21.83	"	0.66	3.03	"	0.10	24.86	plus 59.35	....
ditto plus peas nodule-bacteria	18.17	"	0.53	2.47	"	0.21	20.64	" 32.31	—16.98
ditto plus horse beans nodule-bacteria	20.67	"	1.26	2.87	"	0.41	23.54	" 50.89	— 5.40
ditto plus vetch nodule-bacteria	20.67	"	1.13	2.97	"	0.30	23.64	" 51.54	— 4.97
ditto plus alfalfa nodule-bacteria	19.83	"	0.23	2.73	"	0.18	22.56	" 44.62	— 9.25
ditto plus serradella nodule-bacteria	14.50	"	1.16	1.93	"	0.09	16.43	" 5.32	—33.91
ditto plus lupine nodule-bacteria	17.67	"	0.53	2.33	"	0.06	20.00	" 28.20	—19.55
alfalfa									
uninoculated	3.33	"	0.18	0.27	"	0.05	3.60	....	....
inoculated with alfalfa nodule bacteria	8.33	"	0.53	1.23	"	0.10	9.56	" 165.55	....
ditto plus red clover nodule bacteria	6.50	"	0.20	1.00	"	0.20	7.50	" 108.33	—21.54

Plaintiff's Exhibit 11

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also show how important it is to give careful thought to the selection of the place of clover in the sequence of crops following legumes. And not the least important fact that can be seen from these experiments is that it would be a great mistake to use a universal inoculation material for the various types of legumes. This would never give the same increase in yield as the employment of the particular culture, indigenous to the plant.

846 *Bibliography*

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(2) Heinze, Land. Jahrbuch (German Agricultural Yearbook), 38, 1910, page 340.

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854 PLAINTIFF'S EXHIBIT 12.

DOMINION OF CANADA

DEPARTMENT OF AGRICULTURE

DOMINION EXPERIMENTAL FARMS

DIVISION OF BACTERIOLOGY

PROGRESS REPORT OF THE DOMINION AGRICULTURAL BACTERIOLOGIST

A. G. Lochhead, B.A., M.Sc., Ph. D.

FOR THE YEARS 1931, 1932 AND 1933

Published by authority of the Hon. Robert Weir, Minister of Agriculture, Ottawa, 1934.

855 pasteurized milk in the vat and the same milk after bottling. In addition, surprise samples were taken from time to time to check on the methods employed by the milkers. Results were reported to the Division of Animal Husbandry, from which Division we have received constant co-operation in all our dairy bacteriological investigations.

SOIL BACTERIOLOGY

LEGUME CULTURE DISTRIBUTION

The distribution of cultures for the inoculation of legume seed to Canadian farmers was continued during the years 1931-33. The distribution of "nitro-cultures" is maintained for educational and experimental purposes, sufficient culture for the treatment of 60 pounds of legume seed being sent free to any farmer on the understanding that the user report the results. Our object is therefore (a) to encourage the more widespread practice of seed inoculation and (b) to accumulate data on the practical results of seed treatment under field conditions. During the years under review the numbers of cultures distributed were respectively 2,561, 6,143 and 7,557. The increases noted in 1932 and 1933 were largely due to our co-operation in the scheme of seed loans to farmers of the Prairie Provinces, whereby cultures sufficient to treat the legume seed distributed by Government aid were provided.

By means of reports returned by farmers using cultures, the Division has been accumulating information on the practical benefits accruing from inoculation in the opinion of the farmers themselves who are the ultimate judges of the value of any farm practice. Out of 1,799 reports so far received, 1,403 or 78 per cent indicated a benefit due to inoculation (81 per cent where crops were grown for the first time). In a large proportion of cases where no benefit was noted the treated and untreated crops grew equally well, indicating not a failure of the culture, but rather that the soil was already supplied with the proper strain of bacteria. On the other hand, the information from our reports supports the view that re-inoculation may be of value. The addition of a "good" strain of bacteria, of high nitrogen-fixing capacity or better suited to the soil type, rather than the mere presence of nodule-forming bacteria in the soil, is probably a factor of importance in such cases.

Examination of Miscellaneous Inoculants

During the triennial period a number of legume inoculants appearing on the market were examined at the request of the Seed Branch, Department of Agriculture. The tests undertaken consisted in estimating the numbers of organisms appearing on Ashby's agar, while in addition greenhouse tests were made of the ability of the culture to produce nodules on the appropriate legume plants when applied as directed. Seeds were sown in sterile sand to which was added plant food minus nitrogen with controls of uninoculated seed and of seed inoculated with a pure culture of *Rhizobium* corresponding to that of the inoculant.

While most of the cultures were capable of inducing nodule formation on the appropriate legumes, the results indicated a superiority of the older method, whereby the seed is moistened when the culture is applied, over the dry inoculation method in which the culture, in the form of a finely divided powder is simply mixed dry with the seed. Furthermore cultures containing bacteria for treating legumes of different cross-inoculating groups were unsatisfactory. Where two strains of *Rhizobium* are mixed in a single culture there is the probability of one strain being suppressed, and it is therefore recommended that an inoculant contain bacteria of but one cross-inoculation group.

856 PLAINTIFF'S EXHIBIT 13.

ROOT NODULE BACTERIA AND LEGUMINOUS PLANTS

BY

Edwin Broun Fred
Ira Lawrence Baldwin
Elizabeth McCoy

MADISON
1932

244 University of Wisconsin Studies

857 *Farmogerm culture.* In 1905, Earp-Thomas applied for a patent relating to the growth and distribution of root nodule bacteria and in 1906 secured such a patent (United States Letters Patent No. 816,850). According to the patentee, "The process essentially consists of growing the bacteria in corked and sealed vessels by a nitrogen-free medium for a given period until the organisms reach a stage of exalted virulence." Since that time a number of other patents have been granted him; Nos. 865,965 in 1907; 1,099,121 in 1914; 1,137,388 in 1915; 1,252,332 in 1918; 1,309,723 in 1919 contain further claims and modifications of his process. As in the patent of George T. Moore, United States Letters Patent No. 755,519, 1904, much emphasis is placed on the fact that in the Earp-Thomas culture the organisms are propagated in a nitrogen-free or, as stated in Patent No. 816,850, *substantially nitrogen-free medium*, consisting of water 2210 cc., wood ashes 14 gm., agar 26 gm., and maltose 35 gm.

The claims of these patents might lead one to suppose that the idea of selecting "virulent" organisms originated with Earp-Thomas; however, a mere glance at the literature reveals the fact that Nobbe and Hiltner,

1896c and d; Moore, 1904; and Kellerman and Robinson, 1906; as well as a number of others, had long since realized the value of selecting highly active cultures.

Some idea of the device suggested by Earp-Thomas for permitting the passage of air to cultures, may be gained from a description of his container. In United States Letters Patent No. 1,137,388, of April, 1915, he claimed "A container comprising a closed receptacle, a conduit tube connecting the atmosphere within the receptacle with the atmosphere external to the receptacle and having its inner end reduced and curved circularly and ending and open to the interior of the receptacle at a point removed from the walls of the receptacle, and a plug of fibrous material in the conduit tube."

In 1914 Earp-Thomas proposed the use of sterilized soil for the propagation and distribution of the nodule cultures and also suggested the value of adding other forms of soil bacteria. A later invention, 1918, suggested the use of humus material, inoculated with the desired bacteria, and coated with agar or some protective material. It was claimed that the coating of the particles to which the bacteria are adhering prevents contamination and protects these bacteria from the harmful action of such agents as fertilizers, acids, etc. The granules of peat coated with agar and bacteria may also be mixed with lime, and thus it is possible to inoculate and lime the soil in one operation. According to Manns and Goheen, 1916, Earp-Thomas, 1921 and 1922, and others, humus alone or properly reinforced with nutrient salts offers a very favorable medium for nodule bacteria.

Composite culture. The desire to have one culture for several crops has led to the preparation of the so-called "dual purpose" or "universal culture," Bredemann, 1912, and Vogel, 1917. In some such preparations, rhizobia of only two or three cross-inoculation groups are included, while in others the makers have gone to the extreme of mixing representatives of all of the more common cross-inoculation groups. Although this method may bring about fair nodule formation, the results are variable. The writers feel that composite cultures offer few advantages and certainly many disadvantages. Perhaps the only justification for this mixing of strains is in the case of the small-size garden culture. Because

of the limited amount of seed to be sown, it may be
858 desirable to have one culture containing the organisms for such crops as beans and peas and thus to reduce the cost of inoculation.

Crushed nodule culture. Remy, 1902, attributed the partial failure of Nitragin to an inherent weakness of the culture itself and not to external factors. In order to secure the best results from inoculation, he suggested the use of fresh crushed nodules instead of the pure laboratory cultures. The results of his experimental work clearly demonstrated the superiority of the crushed nodule method of inoculation. Since the report of Remy, Makrinoff, 1913, and others have used cultures prepared directly from nodules, but have found no indication that this type of culture is better than that prepared in accordance with sound bacteriological methods. For example, Ferguson, 1906, at the Virginia Agricultural Experiment Station, sent out cultures for leguminous crops prepared by seeding a suitable medium with a fresh, washed, and crushed nodule taken from the roots of the particular variety of plant to be treated. Undoubtedly such a procedure offers a simple means of preparing cultures; however, there can be no guaranty that the organisms are in a high state of activity and are in any sense superior to the pure cultures kept under regular laboratory conditions. It is needless to say that the Remy method has not found wide favor. Hiltner, 1902, answered Remy's criticism of cultures grown in the laboratory and showed that the nodule organisms do not lose their efficiency as soon as they are removed from the host, but may be grown for years without degeneration if kept on a suitable medium.

Christensen, 1914 and 1916, compared the value of pure culture and crushed nodules for the inoculation of alfalfa. He obtained the best results from the use of the pure culture, although the differences were not great. Because of the perishable nature of the crushed nodule preparations, he maintained that such a method is useless except under local conditions.

Alinit and mixed cultures. From time to time there appear on the market bacterial preparations for use with non-leguminous plants or in some cases for use

with both the Leguminosae and Gramineae. Generally it is claimed by the manufacturer that these cultures intensify the beneficial bacterial processes of the soil, thus bringing about a gain in the available nitrogen of the soil and consequently an increase in the growth of the higher plants.

Caron as early as 1895 reported an increase in yield of non-leguminous plants from the use of pure cultures of an aerobic bacterium. The process of cultivating this organism known as *Bacillus ellenbachensis alpha* was patented by Caron in 1901. The culture was widely exploited under the trade name of "Alinit," and extravagant claims were made concerning its favorable effect on higher plants. Needless to say, the results of careful field and pot tests failed completely to support the claims of the inventor and distributor.

Patents for mixed cultures of bacteria which were supposed to greatly favor plant growth were taken out by Bottomley in 1911c and 1914. In 1924 Earp-Thomas was granted a patent relating to the culture of beneficial soil bacteria, rhizobia, azotobacter, sulphur bacteria, and other forms in a medium composed of green-sand marl mixed with organic food and moisture-retaining material. The names of some of these bacterial preparations follow: Nitro-bacterine, Phosphogerm, Sulphogerm, All-Crop-Farmogerm, Nitrobacter Soil Vaccine, Soilgro,

859 early as 1888 Beijerinck; and Haas and Fred, 1919, pointed out that the germination of the seed of Leguminosae may bring about conditions favorable for the growth of rhizobia. In this connection mention should be made of the effect of age on the reaction of seed. Wilson, 1929b, found that in general the older the seeds the more acid the reaction.

WHAT CONSTITUTES A SATISFACTORY CULTURE OF RHIZOBIA

Just 29 years ago Hiltner and Störmer, 1903a, published the results of their exhaustive and painstaking studies concerning the essential points to consider in the preparation of pure cultures of the nodule bacteria. Briefly these factors are:

1. Purity. The culture should be true to name and free from foreign organisms.

2. Physiological state. To retain the physiological efficiency of the bacteria they should be cultivated on a suitable medium. A gelatin medium is unsatisfactory.
3. Virulence. The organisms must be able to enter the plant and multiply, but must not be so virulent that they act as parasites on the host.
4. Nitrogen-fixing power. The culture of rhizobia should be selected on the basis of its ability to aid the host plant.

Since 1903 the use of artificial cultures has developed rapidly. Unfortunately those in charge of the preparation of these cultures have not always made use of the scientific facts as laid down by the early workers in the field. The proper application of the scientific discoveries would no doubt have prevented much of the disappointment and loss from the use of poor cultures.

To the authors, the value of a culture seems to depend in a large measure on at least five factors.

1. Upon the organism. It is essential that the culture of bacteria contain the proper organism to form nodules on the desired host plant. In general, it is desirable to have only the one type of organism in the culture.
2. Upon efficiency of the strain. Select an active strain of the desired organism. Because of the variation between strains in relation to effectiveness, every effort should be made to select strains of the bacteria which are known to be of decided benefit to the host plant, and to keep these tested cultures under conditions which will maintain the organisms in an active state.
3. Upon the number of bacteria. Just how many bacteria are required in order to produce the maximum number of effective nodules is not known. In general an abundance of the bacteria, probably several thousand per seed, produces the best results.
4. Upon age of culture. Old cultures may be low in total number of viable cells and those which are alive are presumably in a weakened condi-

PLFS. EX. 14 IDEN.

CLOVER

UNINOCULATED STRAIN: 13-19 (UNMIXED) STRAIN: 13-19 (MIXED)

40.4 ±2.0 mg. N 141 ±11.6 mg. N 84. ±3.2 mg. N

402-403

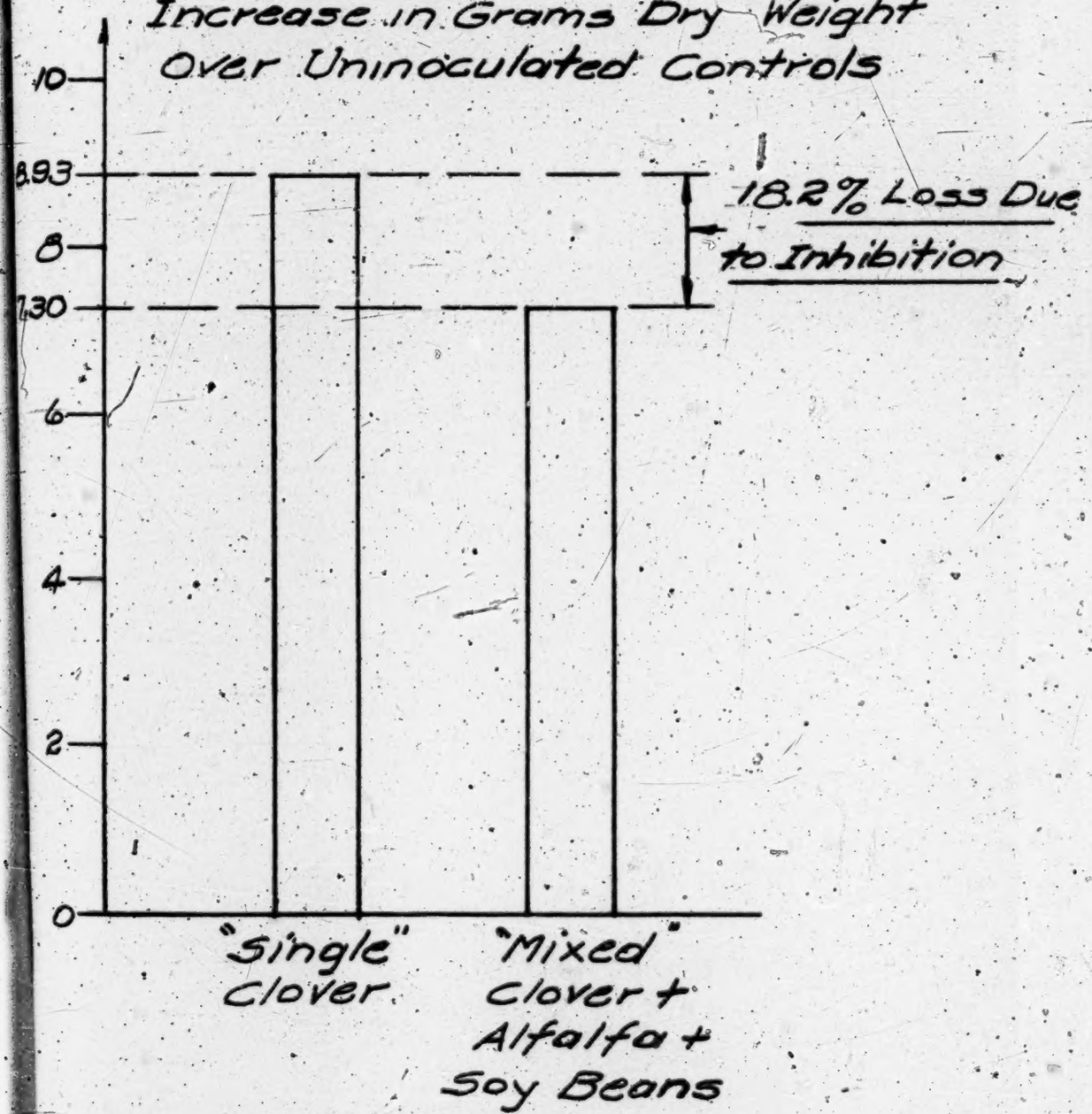
UNINOCULATED ± 8.0 mg. N

STRAIN: 95-96 (UNMIXED) 246 ± 12.0 mg. N

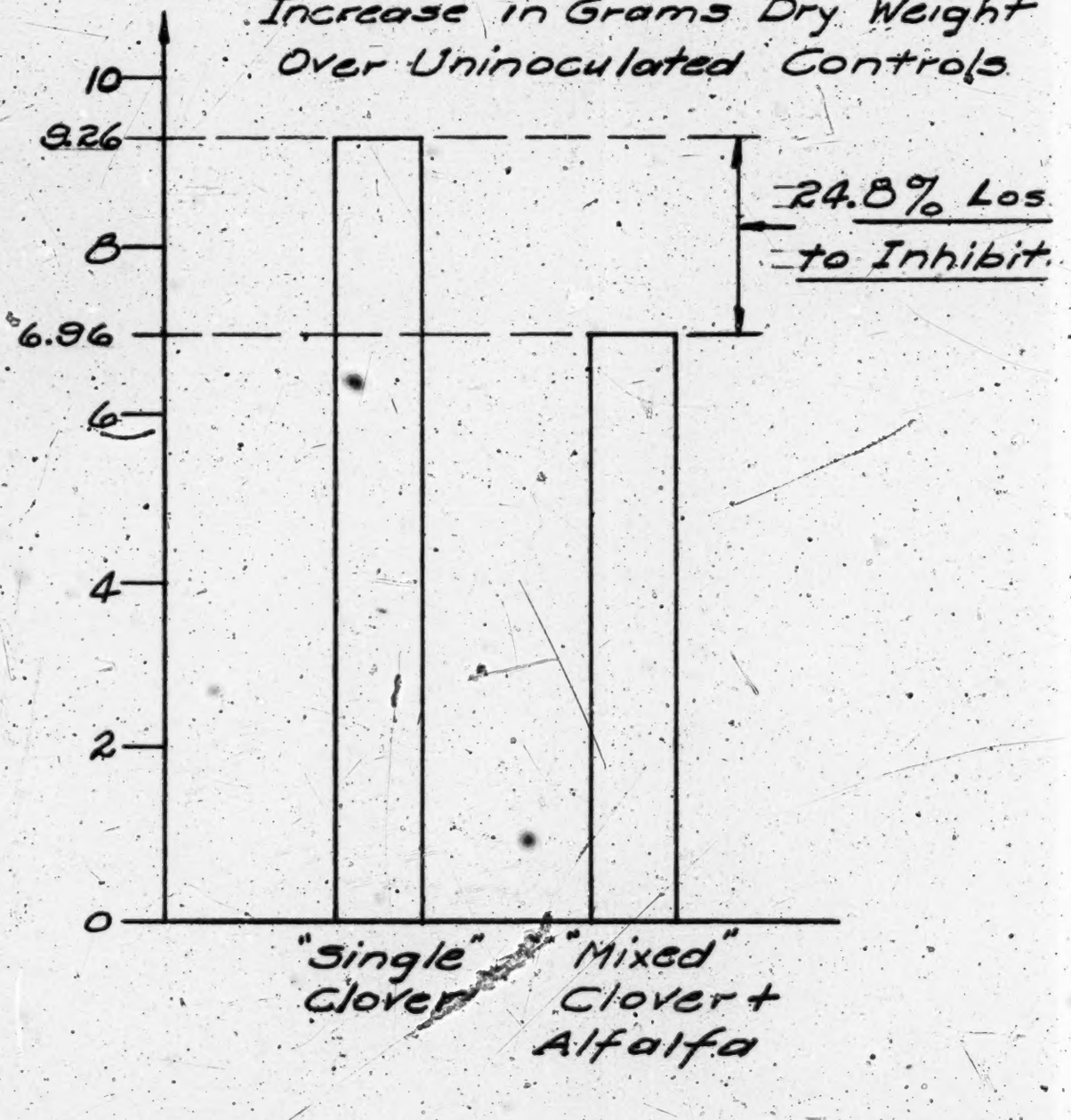
STRAIN: 95-96 (MIXED) 203 ± 11.4 mg. N

404-405

Reduction in Yield of Red Clover Due to Inhibition



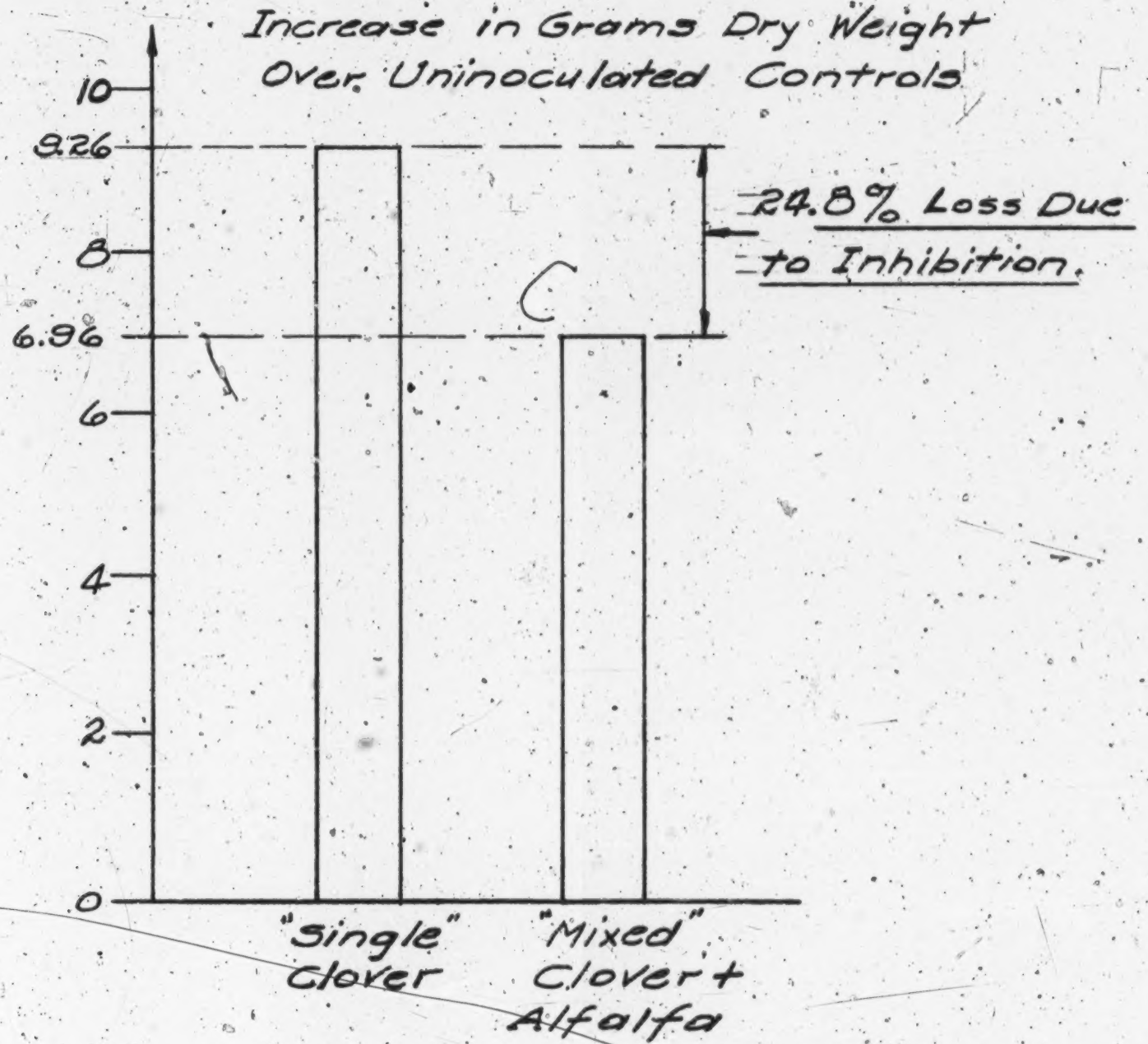
Actual Data from - V.S. Bond.



Actual Data from - Max. Kronberg

PLFS. EX. 16 IDEN.

Clover Due to Inhibition



Actual Data from—Max. Kronberger.

UNI CULTURE

KALO INOCULANT COMPANY

Quincy, Illinois

July 29, 1938

The Nitragin Co., Inc.,
Milwaukee, Wisconsin.

Atten: Mr. L. D. Matchette

Gentlemen:

I can well appreciate that the number of old Nitragin jobbers who have contracted for Uni-Culture, and its proven appeal to dealers, does concern you very greatly. I cannot, however, conceive of anyone sending out such misleading advertising as your bulletin of June 10th or certain letters you have written to jobbers.

You have made no test of Uni-Culture and have no evidence whatever that it is not in fact more effective than Nitragin. You have no knowledge as to how Uni-Culture is produced, but imagine an entirely different method by which you could produce a unit culture and falsely claim it to "be the equivalent of Uni-Culture". You even attempt to support your position with a quotation from a 1932 publication which was not based upon, and does not mention, a unit humus culture for field seed—and in spite of all this you still presume to venture an opinion as to the patent we expect both to secure and to sustain through the courts if necessary.

Under the circumstances we feel you will desire to immediately correct such misunderstandings as have been created by frankly admitting to the seed trade that your arguments have been based only upon the merest conjecture and not on knowledge of the facts.

We would like to hear from you in this connection and hope you will take steps to see that your own letters, the Company advertising, and the statements of the salesmen in the field comply with facts and information definitely in your hands, for we will hold you strictly accountable.

We are photostating this letter and will give it general circulation.

Very truly yours,

KALO INOCULANT COMPANY

MELusk/MA

Pub 9/17

President.

410-411

DIVISION OF BACTERIOLOGY

ROBERT S. BREED, Chief in Research
H. J. CONN, Chief in Research, Soil Bacteriology
G. J. HUCKER, Chief in Research
CARL S. PEDERSON, Chief in Research
M. W. YALE, Associate in Research
ALVIN W. HOFER, Associate in Research
J. R. SANBORN, Special Investigator
H. C. HAMILTON, Resident Doctor
MARY DARROW, Technician
WILLIAM C. HAYNES, Student Assistant
ROBERT F. BROOKS, Student Assistant
MARK FORD, Student Assistant

NEW YORK STATE
AGRICULTURAL EXPERIMENT STATION
GENEVA, N. Y.
P. J. PARROTT, Director

aly 29, 1938.

Mr. V. S. Bond
Kalo Inoculant Company
Quincy, Illinois.

Dear Mr. Bond:

In regard to the letter I sent you the other day there was one thing I omitted to mention. I noticed in a recent publication by Lochhead a statement to the effect that when bacteria of more than one cross inoculation group are placed in the same inoculant, one of the bacteria from these groups sometimes lose their efficiency.

Since this too is a point you will want to check over carefully, I think it wise to call your attention to it.

Yours very truly

Alvin W. Hofer.

A. W. Hofer.

PLFS. EX. 18 IDEN.

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NEW YORK STATE
AGRICULTURAL EXPERIMENT STATION
GENEVA, N. Y.
P. J. PARROTT, Director

July 19, 1938

Mr. B. S. Bond
Kalo Inoculant Company
Quincy, Illinois

Dear Mr. Bond:

Your letter of July 14th is at hand and I was very much interested to read your comments. In general, it seems to me that your comments in regard to my paper fit in very well with what I expressed there. As long as cultures contain 5,000,000 or more bacteria per pound of seed they are to inoculate, I feel confident that the number of organisms will be sufficient. Since the variation in numbers is so enormous, however, it seemed that for a manufacturer to be certain of having 5,000,000 legume bacteria per pound of seed in each of his containers, it would be necessary to try to maintain an average of at least ten times that number. While I have not studied variation in humus cultures to the same extent as in agar cultures, it is my opinion that the numbers of bacteria in humus cultures vary also.

There is one suggestion I should make in regard to your procedure and that is that if possible, you reduce the expiration date. The death of legume bacteria in humus is very rapid after the first two or three months and unless there is a definite need for a distant expiration date, it seems to me that it would be well to move that date forward so that the culture would be marked for good for only six months. Of course I realize that this may not be feasible in the case of Austrian peas and other such legumes. In the case of most cultures, however, the date could be much shorter than it is.

What you say in regard to your new culture combining clover, alfalfa, and soy beans in a single unit is very interesting to me. From the information you have given it seems that this type of culture may be practical. The only question I would have in regard to it would be that of expense. Since it will be necessary to put in an adequate number of each of these types of bacteria, the expense apparently would be much increased but perhaps the advantages of the culture would make up for that. What you state in regard to the advantages of washing agar cultures as a method of preparation of peat cultures has been observed in this laboratory. I think you are on the right track when you add a large number of nodule organisms and a small amount of nutrient material to the humus. I doubt whether there will be antagonism between the different types of legume organisms but you will need to be careful to add sufficient numbers of each type. There is a problem which sometimes occurs in such cultures, in my opinion, and that is that occasionally some of the strains lose their nodule forming ability. You should check on this point very carefully.

It will not be possible for me to be at the S.A. B. meeting this year although Dr. Conn is planning to attend. I do expect, however, to be at the Agronomy meeting in Washington. If you are present I should be very

PLFS. EX. 19 IDEN.

happy to see you and discuss these matters with you further. In fact, it might be worth while for some of us to plan to meet there early since the two days of the microbiological meetings are usually jammed full. It seems to me that you have a promising approach to a problem of legume inoculant manufacture and with the precautions you have already taken, it appears to be possible to make a more uniform type of culture to use. If there are any further developments, I should be very happy to keep in touch with them.

Yours very truly,

Alvin W. Hofer

Alvin W. Hofer

AWH:MC

PLFS. EX. 19 IDEN.

DOMINION OF CANADA
DEPARTMENT OF AGRICULTURE
DOMINION EXPERIMENTAL FARMS

DIVISION OF BACTERIOLOGY

PROGRESS REPORT OF THE DOMINION AGRICULTURAL BACTERIOLOGIST

A. G. LOCHHEAD, B.A., M.Sc., Ph.D.

FOR THE YEARS 1934, 1935, AND 1936

PLPS. EX. 20 IDEN.

Published by authority of the Hon. James G. Gardiner, Minister of Agriculture, Ottawa, 1938

To assist the Division of Animal Husbandry in the control of mastitis in the dairy herd, periodic examinations were made of all cows for infection by *Str. agalactiae*. According to the findings the animals were segregated into definitely infected, suspicious and non-infected groups, which permitted of better control in preventing the spread of the disease and in aiding its elimination.

SOIL MICROBIOLOGY

LEGUME CULTURE DISTRIBUTION

The distribution of cultures for the inoculation of legume seed to Canadian farmers was continued during the years 1934-36. The distribution of "nitro-cultures" was maintained for educational and experimental purposes, sufficient culture for the treatment of 60 pounds of legume seed being sent free to any farmer on the understanding that the user report the results.[1] The object was therefore (*a*) to encourage the more widespread practice of seed inoculation and (*b*) to accumulate the data on the practical results of seed treatment under field conditions. During the years under review the numbers of cultures distributed were 4,184, 5,407 and 5,867 respectively, making a total of 15,458 for the period. Of these, approximately 2,800 were sent to the Prairie Provinces in co-operation with the program of Government aid to farmers in drought areas.

RESULTS OF INOCULATION EXPERIMENTS CONDUCTED BY FARMERS

By means of reports returned by farmers receiving cultures for legume inoculation, the Division has been accumulating information over a period of 12 years on the practical benefits of seed treatment as judged by the farmers themselves, whose opinion will largely determine the value of any agricultural practice. A summary of reports received to the end of 1936 is presented in table 5, comprising data from 2,475 reports.

TABLE 5.—SUMMARY OF REPORTS OF LEGUME INOCULATION BY FARMERS

—	Alfalfa	Sweet clover	Red clover	Alsike clover	Peas	Vetches	Beans	Soy-beans	Lupines	Total
Total reports received	1,449	399	271	74	90	10	53	120	9	2,475
Benefit from inoculation	1,185	294	213	65	67	8	44	82	8	1,976
Percentage benefit	82·6	73·7	79·3	87·8	74·4	80·0	83·0	68·3	88·9	79·9
Legume grown for first time	1,183	311	179	40	53	6	37	108	8	1,886
Benefit from inoculation	1,007	234	107	36	36	5	26	78	7	1,534
Percentage benefit	85·1	75·2	82·3	90·0	67·9	83·3	70·3	72·6	83·8	80·6
Cases reporting no benefit	254	105	56	8	23	2	9	38	1	497
Treated and untreated crops grew well	137	36	20	2	9	2	3	21	-	230
"No catch" reported	38	17	13	1	3	-	3	1	-	76
Winter killed	3	4	1	-	-	-	-	-	-	8
Drought	26	34	12	3	6	-	1	1	-	73
Blown out	1	1	-	-	-	-	-	-	-	2
No reason given	49	23	10	3	6	-	2	15	1	109

From the table it is observed that the results are distinctly favourable to inoculation. There is reason to believe that not only in the case of alfalfa, but also for common clovers and other legumes, seed treatment may be a very useful practice. In approximately one-half of the cases where no benefit was noted, both treated and untreated crops grew well, indicating that the soil was already supplied with suitable bacteria rather than a failure of the culture. Although the percentage of favourable reports was slightly higher where legumes were grown for the first time, information from reports indicated that re-inoculation may be of value. Greenhouse tests, supplemented by field trials at Beaver-

[1] The general distribution was discontinued in 1937, satisfactory commercial cultures now being available.

lodge, have shown that different "strains" of legume bacteria may vary greatly in nitrogen-fixing capacity and so in their ability to aid a crop. The addition of a "good strain" of bacteria rather than the mere presence of nodule-forming bacteria in the soil is believed to be an important factor which explains the value of re-inoculation. (See figure 3.)

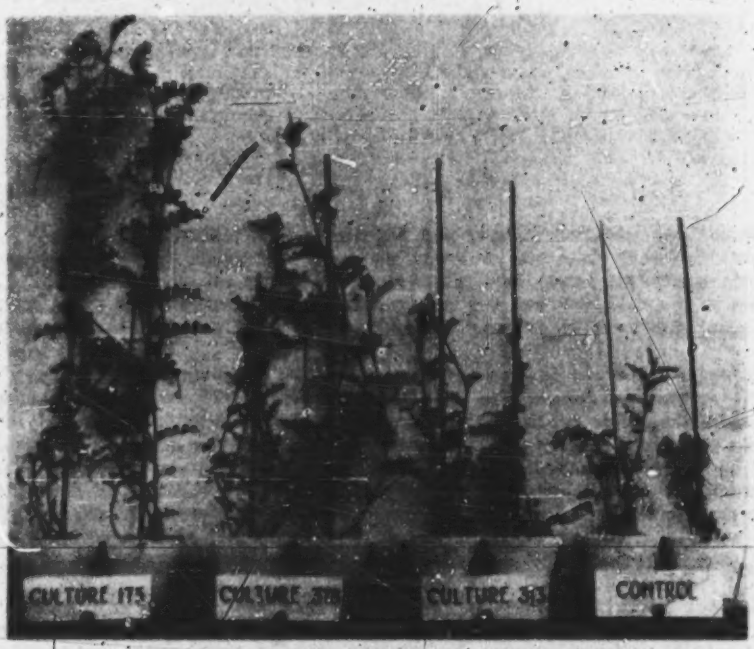

FIG. 3.—Variation in efficiency shown by different strains of nitrogen-fixing bacteria (*Rhizobium leguminosarum*) for vetch.

EXAMINATION OF MISCELLANEOUS INOCULANTS

During the three-year period a number of legume inoculants appearing on the market were examined. The tests undertaken consisted in estimating the number of organisms in the culture and in making greenhouse tests to note the ability of the culture to produce nodules on the appropriate legume plants when applied as directed. Seeds were sown in sterile sand to which was added plant food minus nitrogen with controls of uninoculated seed and of seed inoculated with a pure culture of *Rhizobium* corresponding to that of the inoculant.

Legume inoculants available to farmers in Canada are now believed to be generally reliable. Though various types are available, there is nothing to indicate that the dry method, in which the culture, in the form of a powder, is mixed dry with the seed, is to be preferred to the older method whereby the seed is moistened when the culture is applied. Moreover, inoculants containing bacteria for but one cross-inoculation group are believed better than those containing bacteria for two or more legume groups, since tests have shown that in mixed cultures one strain may become ineffective.

May 18, 1945

S A variation,

Dr. [redacted]
N. J. Agricultural Exp. Station
[redacted]
New Brunswick, N. J.

Dear Dr. Conn:

You may have heard of the lawsuit now pending between two inoculant companies, and which is scheduled to go to court in the near future. I am told that it will be necessary for me to testify, and since certain questions are involved that deal with problems with which you are familiar, I am wondering whether you would be willing to give me the benefit of your opinions.

Briefly, the question at issue revolves around certain statements in the patent. One is: "It has heretofore been considered impracticable to prepare a composite culture inoculant containing organisms which will cause nodulation on more than one of the cross-inoculation groups. This has not been done because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen fixation by the plant and the organism was inhibited or even prevented. For this reason, it has been considered desirable to use a culture containing a single species of organism rather than on containing a mixture."

Also, "As has been pointed out above, composite cultures for inoculating the seeds of leguminous plants have not generally come into use because of the inhibitory effect that certain strains of certain species of the Rhizobia have on each other."

A good deal of the case hinges around this question of inhibition of nitrogen fixation in one group by bacteria of a different group. Because of the importance of this point, I should appreciate knowing whether or not you believe that such an effect actually occurs.

The more answers there are to this letter, obviously, the better will be the measure of scientific opinion on the subject. Unless you object, it is my plan, if and when I am called, to include your answer with the evidence.

Assuring you of my great appreciation for any support you may give me in presenting this matter fairly, I am

Sincerely yours,

A. W. HOFER

AWH:B

From Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten (German Central Journal for Bacteriology, Parasitology, and Infectious Diseases), part II, vol XX, 1908, pp 61-71.

Reprinting prohibited.

Observations on the Action of Hiltner's Pure Cultures for Legumes.

(Report of Kaiser Wilhelm Institute for Agriculture, Bromberg, Germany).

By Prof. Dr. Gerlach and R. Vogel

With 3 figures

Part I

For the purpose of testing the action of Hiltner pure cultures on the development of serradella, lupines and soya beans, these plants were, in the spring of 1906, brought under cultivation in walled-in parcels with and without inoculation. The parcels were prepared in November 1905, that is, in such a way that the ground was first dug out at the places concerned to a depth of 1 meter. The ditches thus formed then received vertical brick walls of 1 meter height, after the removed soil was put back in at the same place from which it had been removed. There were thus obtained square parcels, open below, that is, in contact with the natural ground, of 1 sq. m. in area and 1 cu. m. in volume. The parcels line in the range of the drainage system of the experimental fields, by which system they were drained. The soil consists of humus sand resting on light-yellow sand. At a depth of about 2 meters there is encountered an impermeable clay layer. The soil contains:

0.092% of nitrogen
0.061% of phosphoric acid
0.025% of potassium
871 0.590% of total lime

Mechanical analysis showed:

gravel	0.4%
very coarse sand	1.6%
coarse sand	5.8%
medium-coarse sand	48.2%
fine sand	35.6%
erosive parts	8.4%
	100.0%

The earth which had been put back into the walled trenches remained lying during the winter of 1905-1906 in rough furrows. Whether legumes, particularly lupines and serradella had ever been cultivated in this ground, we do not know. In the previous 4-5 years, in any event, this was not the case, for the ground had lain fallow during this period, and carried an exceedingly luxuriant stand of weeds. The experimental arrangement can be seen from the following sketch:

1	2	3	4	5	11	12	13	14	15	21	22	23	24	25	uninoculated
6	7	8	9	10	16	17	18	19	20	26	27	28	29	30	inoculated
	serradella					yellow lupine					soya bean				

On April 4, 1906 each of the 30 parcels received a fertilization consisting of 12 grams of potassium in Kainit (equals 120 kg of potassium per hectare) and 10 grams of citric-acid solution in Thomas flour (equals 100 kg of phosphoric acid per hectare). Each of the parcels allocated for the cultivation of soya bean, 21-30, was also fertilized with 500 grams of lime carbonate (equals 5000 kg per hectare). On April 19th the serradella was sown in the parcels 1-10 and the lupine in the parcels 11-20. The soya bean was sown on May 3rd (1). The quantities of seed amounted to:

Serradella, 5 grams per parcel, scattered by hand, and raked in.

872 Lupine, 20 grams per parcel, divided into 4 rows each.

Soya bean, 20 grams per parcel, likewise divided into 4 rows each.

As can be seen from the sketch, the parcels 1-5, 11-15, and 21-25 received the uninoculated seeds and the parcels 6-10, 16-20 and 26-30 the corresponding uninocu-

lated seeds. Each of the inoculations occurred in such a way that the pure culture, together with the corresponding nutrient salts, were distributed in 1/4 liter of fresh milk, and the seeds were thoroughly wetted with the bacteria liquid thus obtained. To the serradella seed there was preliminarily added a small amount of earth from the corresponding parcels for the purpose of enabling a more uniform broadcasting. The sowing of these seeds occurred by broadcasting, while the lupine and soya beans were placed in rows, and, in the same way as the serradella seed, immediately covered with earth. The uninoculated seeds were, for the purpose of establishing the same conditions, wetted with an equal quantity of nutrient salts added with milk, and sown in the same way. The weather was very favorable in sowing the serradella and lupine, with an overcast sky. The next day it rained. When the soya beans were sown the sun was shining. Since the spring and summer of 1906 were dry, all of the parcels were sprinkled from time to time with tap water. The stand of the inoculated lupines was already better than that of the uninoculated by the beginning of June. They were colored a deep darkgreen, and were far developed. Plants of the inoculated parcels pulled out at random exhibited well developed nodules. On the uninoculated plants these were entirely absent. By June 10th there was present a pronounced difference in favor of the inoculated parcels, including the serradella. The inoculated plants had now acquired a darkgreen color, while the uninoculated serradella had a light-green appearance and
873 was retarded in growth. In the further course of vegetation the lupine was somewhat injured by larvae and the lupine fly. The number of sick plants was greater on the uninoculated than on the inoculated parcels. On June 14th each third plant of the uninoculated and inoculated parcel was pulled out by the roots. The former were entirely free of nodules, while the latter exhibited very well developed nodules, particularly on the top parts of the main roots. On June 25th the inoculated lupines exhibited a copious supply of buds, in which the yellow color of the flower could be seen. On the uninoculated plants there could not yet be observed the beginning of blooming. Toward the end of June there became very noticeable in all of the experi-

ments, among the uninoculated, weakly, pale-green plants, individual examples of dark-green, luxuriantly-developed plants, which exhibited a copious coverage of nodules. There must, therefore, either have been present in the soil of the experimental field active nodule bacteria, or there must have occurred, despite the greatest care, an infection of these parcels in the inoculation. On July 12th the lupines were harvested by cutting off the plants above the ground. They had already dropped most of their blossoms and acquired larger numbers of pods. The individual yields can be seen from the following table, from which there can be recognized considerable increases in yield due to the inoculation.

The tabulated values show that the yield of the uninoculated parcels was subject to greater variations than that of the inoculated parcels. In the former case there were present differences up to 1003 grams, but in the latter only up to 330 grams. Of both the inoculated and the uninoculated parcels we took 30 plants each for further study of the nodule conditions. The roots of all of the inoculated plants exhibited strongly developed nodules, nearly all of which were present at the top part of the main root. Of the 30 roots of the uninoculated

874

parcel No.		weight of the above-ground, green mass, grams	total yield, grams
11	uninoculated	2505.0	10,715.0
12		1500.9	
13		2110.0	
14		2500.0	
15		2100.0	
16	inoculated	3200.0	15,520.0
17		3070.0	
18		2900.0	
19		3120.0	
20		3230.0	

plants only three exhibited nodules. In 2 cases the nodules were present in the form of large spherical swellings on the bottom part of the main root, and in one case the main root was free of nodules while a number of nodules were present on a branch root. The remaining 27 roots of the uninoculated plants were entirely free of nodules. It is possible, and becomes very probable through the experiments described below,

that these nodules, occurring on the bottom part of the main root or on the branch roots, were caused by bacteria previously present in the soil. The nodules of the uninoculated plants of the parcels 11-14, which had been so outstanding through their luxuriant stand, were, conversely, as in the case of all of the inoculated plants, present on the top part of the main root. In this case, therefore, there must, as already mentioned, have occurred an infection through the inoculating bacteria. Surprising was the retarded root development of the inoculated plants.

On July 13th the 10 lupine parcels were, in observing the necessary precautions (using separate utensils for the inoculated and uninoculated parcels, etc.), scraped-flat, and, without reinoculation, again planted with lupine. Also this time there occurred clear differences in the development of the plants in favor of inoculation, which were clearly expressed in the harvest yields. Harvesting occurred on October 11th. The yields can be seen in the following tables:

875

parcel No.		weight of the above-ground, green mass, grams	total yield, grams
11		1029.0	
12		865.0	
13	uninoculated	918.0	4849.0
14		1001.0	
15		1036.0	
16		1645.0	
17		2028.0	
18	inoculated	1730.0	8364.0
19		1586.0	
20		1375.0	

There had thus been obtained in the form of green mass:

	uninoculated	inoculated
I. harvest	10,715.0 g	15,520.0 g
II. harvest	4,849.0	8,364.0
	15,564.0 g	23,884.0 g

The serradella of the 10 experimental parcels were harvested on August 8th, that is, again by cutting off the green mass above the ground. The harvest results were as follows:

parcel No.		weight of the above-ground, green mass, grams	total yield, grams
1		1612.0	
2		1586.0	
3	uninoculated	1681.0	8530.0
4		1931.0	
5		1720.0	
6		2179.0	
7		1795.0	
8	inoculated	1967.0	9985.0
9		1904.0	
10		2140.0	

This gives per parcel:

uninoculated	inoculated
1706.0 grams	1997.0 grams

of green mass.

All of the roots of the inoculated plants exhibited large masses of nodules, in many cases sitting together in the form of thick nests on the main root. With 876 the uninoculated plants, large numbers of nodules were present on about 50% of the roots, although these were mostly present on the branch roots.

With the soya beans the results of inoculation were apparently the least pronounced. They were ripe by September 15th, when they were harvested together with the roots. The yields can be seen from the following table:

parcel No.		weight, grams	total weight, grams
21		759.0	
22		777.0	
23	uninoculated	843.00	4259.0
24		970.0	
25		1010.0	
26		891.0	
27		1157.0	
28	inoculated	932.0	5676.0
29		1290.0	
30		1406.0	

Thus the yield per parcel was:

uninoculated	inoculated
871.0 grams	1135.2 grams

The roots of the uninoculated plants were entirely free of nodules. However, also in the inoculated par-

cels there were some plants whose roots had no nodules. The plants were kept for further drying in paper bags, after which the grain was removed from the stems. There were obtained:

from parcels 21-25 (uninoculated), 515 grams of grain, 1713 grams of straw

from parcels 26-30 (inoculated), 635 grams of grain, and 2031 grams of straw

In all cases, therefore, an increase yield was obtained through inoculation. All of the results are recapitulated in the following tables:

877 **Serradella**

(1) No.
(2) parcel No.
(3) treatment
(4) harvest per parcel
(5) nitrogen-content of green mass, percent
(6) harvested dry substance per parcel, grams
(7) harvested nitrogen per parcel, grams

1	2	3	4	5	6	7
1	1-5	uninoculated	1706.0	0.399	395.79	6.81
2	6-10	inoculated	1997.0	0.572	479.28	11.42

Yellow Lupine

(1) No.
(2) Parcel No.
(3) treatment
(4) harvest per parcel
  (a) 1st harvest
  (b) 2nd harvest
  (c) total.
(5) nitrogen-content of green mass, percent
  (a) 1st harvest
  (b) 2nd harvest
(6) harvested dry substance per parcel, grams
(7) harvested nitrogen per parcel, grams

1	2	3	4			5		6	7
			a	b	c	a	b		
3	11-15	uninoculated	2143.0	969.8	3112.8	0.267	0.353	427.28	9.15
4	16-20	inoculated	3104.0	1672.8	4776.8	0.436	0.448	656.34	21.03

878 **Soya Bean**
(1) No.
(2) parcel No.
(3) treatment
(4) harvest per parcel
(a) grain
(b) straw
(5) nitrogen-content, percent
(a) grain
(b) straw
(6) harvested dry substance per parcel, grams
(7) harvested nitrogen per parcel, grams

1	2	3	4		5		6	7
			a	b	a	b		
5	21-25	uninoculated	103.0	342.6	4.33	0.579	407.93	6.44
6	26-30	inoculated	127.0	406.2	4.40	0.580	481.52	7.94

At the same time inoculation gave the following increase in yield:

	dry substance, %	nitrogen, %
with lupine	53.6	129.9
with serradella	21.1	67.8
with soya bean	18.0	23.3

This was supposed to end the experiment, to be followed the next year with a watering experiment on lupine. The harvested parcels were dug around in the autumn, and were left lying over winter in the form of rough furrows. In the spring of 1907 we planted all of them with yellow lupine, all of which had been inoculated with Hiltner's pure culture. All of the parcels received on
879 March 21st a fertilization with potassium-phosphate. The sowing of the inoculated seed occurred on March 28th. During the growth of the lupine we now made the following interesting observations. As early as June 5th it was noticed that the lupine in parcels 1-10, which had carried serradella the previous year, exhibited a considerably stand than those in which there had previously grown lupine and soya bean. The plants were a deep dark-green and gave, as compared with the other lupines, the impression of plants which had been successfully inoculated. On June 18th there bloomed the lupines in the parcels 1-10 (previous crop, serradella); the stand was exceptionally thick, and the plants a deep dark-green. Of the considerably worse developed plants of the other parcels not one had yet bloomed. In the parcels 11-20 (previous crop, lupine), however, some of the plants had

begun to green and to develop vigorously. By far the worst was the stand in the parcels 21-30 (previous crop, soya bean).

On June 25th the lupines in all of the parcels were in full bloom, the best in the parcels 1-10 and the worst in the parcels 21-30. Measurements gave the following values:

average length of plants with roots

lupine from parcels 1-10 (previous crop, serradella), 55-60 cm

lupine from parcels 11-20 (previous crop, lupine), 40-45 cm

lupine from parcels 21-30 (previous crop, soya) 15-25 cm

On July 8th there were taken 12 plants each of the parcels of each of the groups for investigation. These weighed:

lupine from parcels 1-10 (previous crop, serradella), 705 grams

lupine from parcels 11-20 (previous crop, lupine), 265 grams

lupine from parcels 21-30 (previous crop, soya), 135 grams

The lupines following serradella were 65-75 cm long, and all were supplied with nodules, all of which were present on the top part of the main root, where they had brought about thick swellings. Only one of the 12 880 plants investigated also exhibited nodules on the branch roots, but also here on the top part. Of the lupines following lupines some exhibited nodules and some did not. The former possessed a length of about 58 cm and the latter one of 42 cm. Of the 12 plants, 7 had no nodules whatever. In the remaining 5, strongly-developed, thickly-projecting nodules were present, besides on the top part of the main root, also on the bottom part and on the branch roots. Nearly all of the lupines following soya seem to be light-green and sickly. Only a very few of the plants exhibited a better dark-green color. All 12 of the investigated plants were without nodules, and had an average length of 42 cm. In these plants the roots were longer and hence the above-ground plants shorter than the previous ones. One of the better-developed, dark-green plants exhibited a large nodule the size of a hazel

nut on the bottom part of the main root, as well as a few nodules on the branch roots.

On July 9th there were again taken 12 plants from each of the groups. The weights amounted to:

in group I, (previous crop, serradella), 675 grams
in group II (previous crop, lupine), 532 grams
in group III (previous crop, soya), 320 grams

The weights in groups II and III are comparatively high because there were purposely selected plants of a dark-green color and a good development, which were to serve for further study of the nodule conditions. This study gave the following picture. All of the roots of group I (see figure 1) exhibited the nodule joined in the form of bulbous swellings at the topmost part of the main root. The whole root system, as well as the plant, was very vigorously developed. The branch roots were without nodules in nearly every case.

Quite different in group II. Here infection had, obviously owing to less active bacteria, and also, as may be concluded from the later greening of the plants, set in 881 later. The whole root system here was more weakly developed. The nodules (see figure 2, No. 1, 2, 5, 6) were almost exclusively present on the branch roots. The nodules observed on the main root did not, in some of the cases (see No. 3, 4), sit on the topmost part, and did not, what we regard as particularly typical owing to its very constant repetition, form, like the very infective bacteria of group I, thick, bulbous, kidney-shaped swellings, but thickly-bulging balls. Such nodules present on the middle or bottom part of the main root would seem to be less active. This is confirmed by the picture presented by the roots of the plants of group III with their sparse supply of nodules. These lupines are the worst developed. As can be from No. 1 of figure 3, there is present on the middle part of the main root a spherically-bulging nodule which has been brought about by a virulent bacteria. Such nodules are also present on the branch roots (No. 2), but never on the top part of the main root. By far the majority of these plants of this group, however, fail to show any nodules on the roots (No. 3-6), as a result of which they present a very sickly appearance.

On July 11th there were again taken 12 plants of each of the groups, for the purpose of isolating the nodule bacteria. The weights amounted to:

Figure 1: serradella-lupine

Figure 2: lupine-lupine

Figure 3: Soya-lupine

12 plants of group I (previous crop, serradella), 638 grams
12 plants of group II (previous crop, lupine), 578 grams
12 plants of group III (previous crop, soya), 344 grams

882 On the plant roots the nodules are again distributed in the typical manner already described.

On July 12th the lupines were harvested. The plants were cut off right above the ground, so that the roots remained in the ground.

Group I (previous crop, serradella)

previous year

uninoculated		inoculated	
parcel 4, 3500 grams		parcel 9, 3435 grams	
parcel 5, 3950 grams		parcel 10, 3270 grams	
	7450 grams		6705 grams

7450 grams
6705 grams
2018 grams (harvested earlier)

16,173 grams equals 4043 grams per parcel

Group II (previous crop, lupine)

previous year

uninoculated		inoculated	
parcel 11, 2690 grams		parcel 16, 2850 grams	
parcel 12, 1334 grams		parcel 17, 2235 grams	
parcel 13, 842 grams		parcel 18, 1977 grams	
parcel 14, 1412 grams		parcel 19, 1922 grams	
parcel 15, 2032 grams		parcel 20, 2100 grams	
	8310 grams		11,084 grams

8,310 grams
11,084 grams
1,375 grams (harvested earlier)

20,769 grams equals 2077 grams per parcel

Group III (previous harvest, soya)

previous year	uninoculated		inoculated	
883	parcel 21,	632 grams	parcel 26,	683 grams
	parcel 22,	517 grams	parcel 27,	448 grams
	parcel 23,	815 grams	parcel 28,	725 grams
	parcel 24,	634 grams	parcel 29,	739 grams
	parcel 25,	595 grams	parcel 30,	602 grams
		3193 grams		3197 grams

3193 grams
3197 grams
799 grams (harvested earlier)

7189 grams equals 719 grams per parcel

Investigation of the harvested lupines gave:

lupine following	water	nitrogen
serradella	85.3%	0.450%
lupine	84.7%	0.449
soya	81.4%	0.275%

From this there is calculated:

	per parcel, grams.	
lupine following	dry substance	nitrogen
serradella	594.32	18.19
lupine	291.31	8.55
soya	133.73	1.96

The values bring out clearly the fact that the lupines following serradella have the highest yield of green mass, dry substance and nitrogen. There then follow those following lupine.

Still much lower is the harvest yield of the lupines following after the soya beans cultivated the previous year. This peculiar behavior of the lupines of this year's lupines becomes still more interesting through the circumstance that the seeds of all 30 the parcels were exactly the same kind, and had been inoculated with the same cultures, again furnished by Hiltner, which had had such a favorable effect on the development of the lupines the previous year. It would have been expected that the lupines
884 of this year in the lupine parcels of last year would give the highest yield, after which there would come the lupines following serradella. The inoculating bacteria in group II remained nearly inactive, as may be concluded from the large number of plants which remained without nodules. In group III they suffered a direct injury. In view of the perfectly uniform composition and

treatment of the soil of the 30 parcels it must, therefore, be assumed that the organism flora of the soil had been influenced through the legumes of the previous year in such a way that following serradella there predominated lupine bacteria acting very favorably on lupine, less favorably following lupine, and injuriously following soya. The development following serradella is excellent, no weakly-developed plants whatever being present. The bacteria present in the soil did not, therefore, harm the inoculating bacteria in any way, or else they are exceptionally active for lupine. Following lupine there did not occur the good action of the bacteria which was to be expected. This was even much less the case with group III. Here no inoculation effect occurred. The inoculation bacteria, supplied the previous spring, have been, under the unfavorable conditions prevailing following soya, either completely destroyed or rendered wholly inactive.

From a large number of typical nodules of the roots of groups I, II and III there have been obtained nodule bacteria in the pure culture. We shall undertake, in the spring, with the breeds obtained, inoculation experiments under the most various conditions.

In the isolation of the nodule bacteria from the root nodules of the various plants there was disclosed the peculiar and interesting phenomenon that the bacteria of the lupines grown following serradella, which had been regarded as particularly active, only grew retardedly and slowly in the artificial lupine nutrient soil (lupine agar according to Hiltner), while the bacteria of the crop rotations of lupine-lupine and soya-lupine grew luxuriantly right from the start. This behavior is wholly analogous to that of the pathogenic bacteria which usually thrive in artificial nutrient soil the better the more they have lost in virulence. It will attempt to favor the growth of the serradella-lupine bacteria through the addition of serradella root extract to the lupine nutrient soil.

885

We think that these experiments indicate that the bacteria of serradella and lupine are able to substitute each other, a fact which is in agreement with the opinion recently expressed by Hiltner concerning the homogeneity of nodule bacteria.

We shall endeavor to ascertain, through further experiments, the reason for the serious injury of the inoculating

bacteria for lupine grown after soya bean, as well as of the weak or low activity of these bacteria following lupine.

The above-mentioned watering experiments planned for 1907 have suffered a great deal through the peculiar behavior of this year's lupines. Nevertheless, the values found are so interesting that we give them below.

1907 was a very poor year in the province of Posen for watering experiments, since the precipitation during the summer was much higher than normal. There fell in the experimental fields in Bromberg:

	summer of 1907	normal precipitation (average of 1861-1895)
May	82.5 mm	45 mm
June	55.7 mm	62 mm
July	122.3 mm	62 mm
August	40.5 mm	55 mm
	201.0 mm	224.0 mm

Despite this, a small amount of watering even considerably increased the yield of lupine following serradella, while with the lupine following lupine and soya the watering had an injurious instead of a beneficial effect.

886		dry substance, grams	nitrogen, grams
Lupine following serradella			
	through natural precipitation	790.9	25.78
	ditto plus 150 mm of watering	1023.3	31.25
	ditto plus 300 mm of watering	1067.1	31.52
Lupine following lupine			
	through natural precipitation	273.1	8.01
	ditto plus 150 mm of watering	211.4	6.21
	ditto plus 300 mm of watering	255.4	7.48
Lupine following soya			
	through natural precipitation	143.2	2.10
	ditto plus 150 mm of watering	127.8	1.88
	ditto plus 300 mm of watering	111.4	1.64

The experiments show very clearly that the amount of water supplied to lupine—and the same thing is also true of the nutrient substances—cannot be properly and fully utilized unless the soil contains a sufficient amount of active nodule bacteria.

Footnote

(1) The soya beans used in the experiments, as well as all of the inoculating cultures, were made available to us through the friendly cooperation of Dr. Hiltner, State Councilor, Munich.

(Translated by Carl Demrick)

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ALFALFA, WHITE SWEET CLOVER, YELLOW SWEET CLOVER, BUR CLOVER, HUBAM CLOVER	2	
		—GARDEN SIZE— FOR PEAS, BEANS, SWEET PEAS AND LIMA BEANS. For 10 lbs. Seeds (3) 50c.
ALL VETCHES, CANADA FIELD PEAS, BROAD BEANS, SWEET PEAS, PERENNIAL PEAS, GARDEN PEAS	3	EACH CULTURE HAS BEEN SCIENTIFICALLY SELECTED FOR THE PARTICULAR GROUP OF SEEDS FOR WHICH INTENDED.
GARDEN PEAS, GARDEN BEANS, SWEET PEAS, ANY VARIETY	5	
COWPEAS, PEANUTS, LIMA BEANS, VELVET BEANS	7	Cultures (5), (6), (7), (8) and (9)—Large Seeds: ½ lb. 30 lbs. .30 · 1 lb. 60 lbs. .45 · 2 lbs. 120 lbs. .75 · 5 lbs. 300 lbs. 1.50 · 20 lbs. 1200 lbs. 5.00
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SOY BEANS, ANY VARIETY	9	

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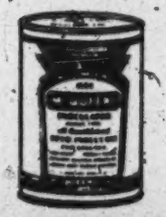

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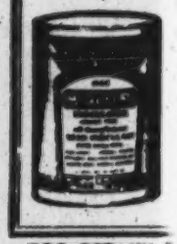

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1 lbs.	2 lbs.	.50
2½ lbs.	5 lbs.	1.00
	10 lbs.	1.85
	20 lbs.	2.50

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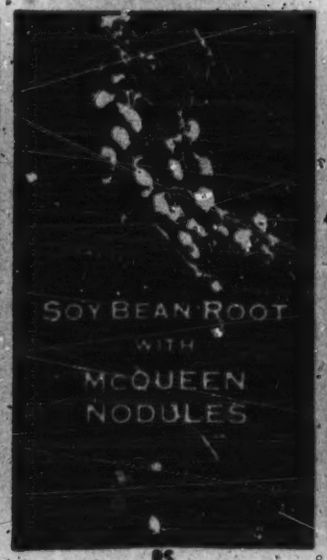

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	10 lbs.	1.00	1.85
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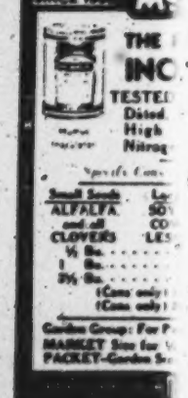

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INOCULATOR
For 15 Lbs. of SEED - 25c
Insures High Yields and Best Quality.
Billions of Bacteria
For Inoculating Garden Peas & Beans Sweet Peas, Lupines and Lima Beans.

FOR RETAIL CIRCULARS "M" CATALOGS & NEWSPAPERS

UEENS
AST-SELLING
CULATOR
AND APPROVED
Protected Package,
Count, Efficient
n-Fixing Bacteria.

S WHOLESALE
A PRICES RETAIL
..... $ .10 $ .20
..... .20 .30
..... .40 .60
..... 1.40 2.00
..... 1.70 2.50

Y NAME OF SEED

"P" PRICE LISTS & CATALOGS

McQUEENS
THE FAST-SELLING
INOCULANTS
TESTED AND APPROVED
Dated, Protected Package,
High Count, Efficient
Nitrogen-Fixing Bacteria.

Small Seeds ALFALFA and all CLOVERS	Large Seeds SOY BEANS COW PEAS LESPEDEZA	WHOLESALE PRICES	RETAIL
½ lbs.	1 lbs.	$ .10	$ .20
1 lbs.	2 lbs.	.20	.30
2½ lbs.	5 lbs.	.40	.60
	(Cases only) 10 lbs.	1.40	2.00
	(Cases only) 20 lbs.	1.70	2.50

Garden Groups: For Peas, Beans, Sweet Peas, Lima Beans
MARKET Size (not ½ lbs. per doz. $2.40 each 20c
PACKET-Garden Size per doz. .20 each 5c

FOR WHOLESALE "W" PRICE LISTS & CATALOGS

NDY ORDER CARD

Defendant's Exhibit 5.

893 EFFICIENCY OF CLOVER BACTERIA AS AFFECTED BY ALFALFA BACTERIA.

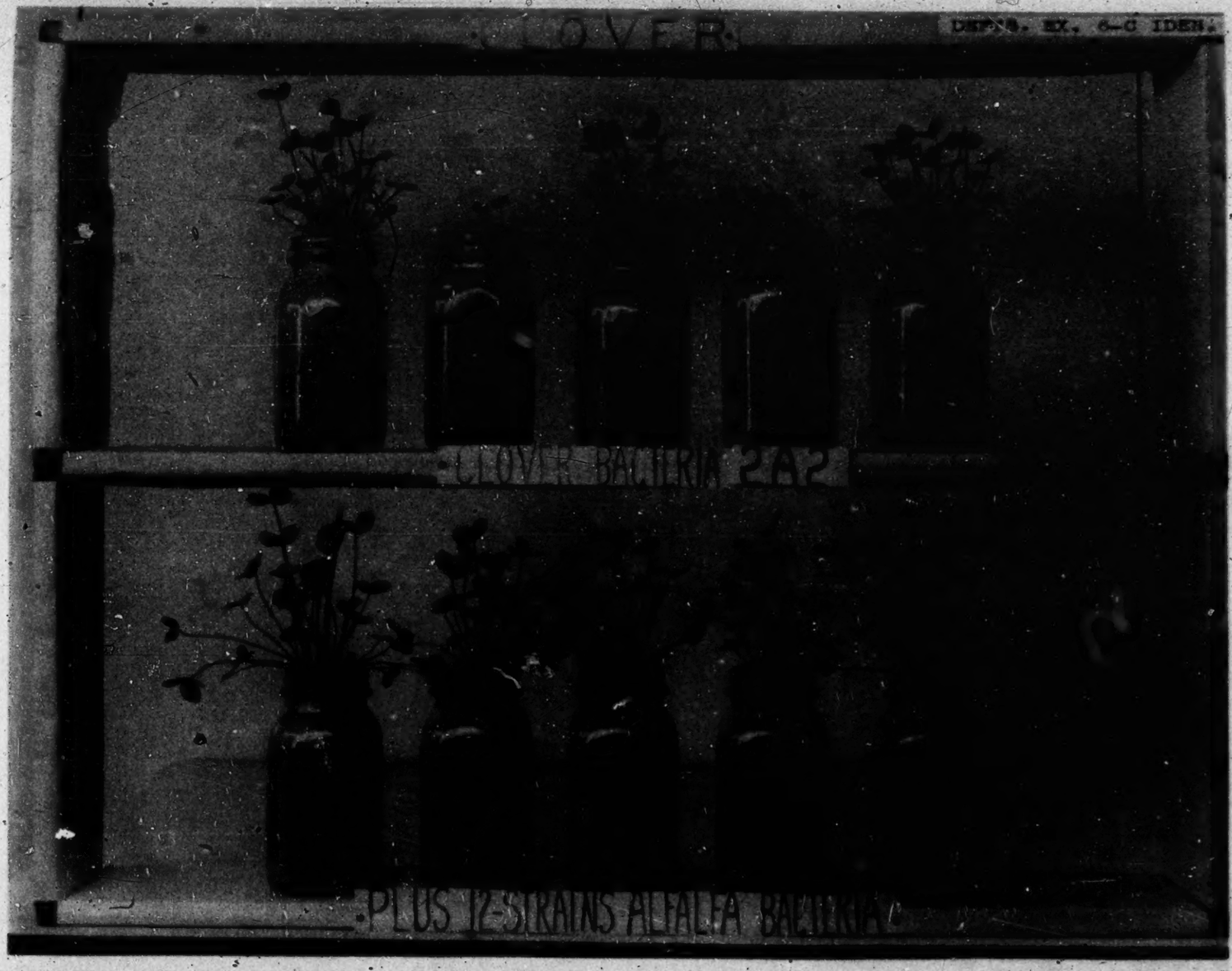
October 27, 1944

Strain	Weight Per Jar	Effect of Alfalfa	Nitrogen	Effect of Alfalfa
201	556 mgm.		21.8 mgm.	
201 Alfalfa	506	— 50	19.8	— 2.0
202	760		32.2	
202 Alfalfa	740	— 20	31.4	— 0.8
2A1	566		24.6	
2A1 Alfalfa	752	+186*	31.4	+ 6.8
2A2	334		14.6	
2A2 Alfalfa	728	+394*	32.0	+17.4*
2A3	650		27.8	
2A3 Alfalfa	[illegible]	— 90	22.6	— 5.2
2A4	292		13.2	
2A4 Alfalfa	654	+362*	28.8	+15.6*
2A5	280		11.2	
2A5 Alfalfa	652	+372*	27.2	+16.0*
2A6	316		13.6	
2A6 Alfalfa	710	+394*	29.6	+16.0*
2B1	520		22.2	
2B1 Alfalfa	788	+268	33.4	+ 9.2
2B2	538		21.8	
2B2 Alfalfa	694	+156	29.2	+ 7.4
2B3	620		25.6	
2B3 Alfalfa	752	+132	32.0	+ 6.4
2B4	316		13.4	
2B4 Alfalfa	722	+406*	30.0	+16.6*
All Clovers Mixed	364		15.8	
Clovers & Alfalfa	1020	+656*	41.8	+26.0*
AVERAGES				
Clovers alone	479		20.2	
Clovers & Alfalfa	688	+209	28.1	+ 7.9

* Difference statistically significant.

DEPTS. EX. 6-A IDEN.
BACTERIA 202
PLUS 12-STRAINS ALFALFA BACTERIA
447-448

DEFTS. EX. 6-B IDEN.
OVER.
PLUS 12 STRAINS ALFALFA BACTERIA.
449-450


CLOVER
DEFNS. EX. 6-C IDEN.
CLOVER BALTIKA 2 A2
PLUS 12 STRAINS ALFALFA BALTIKA C

451-452

CLOVER
DEFTS. EX. 6-D IDEN.
CLOVER BACTERIA 2A3
PLUS 12 STRAINS ALFALFA BACTERIA
453-454

OVER
DEPTS. EX. 6-E IDEN.
CLOVER BACTERIA 2A4
PLUS 12-STRAINS ALFALFA BACTERIA

455-456

CLOVER
DEPTS. EX. 6-F IDEN.
CLOVER BACTERIA 2A5
PLUS 12-STRAINS ALFALFA BACTERIA

457-458

459-460

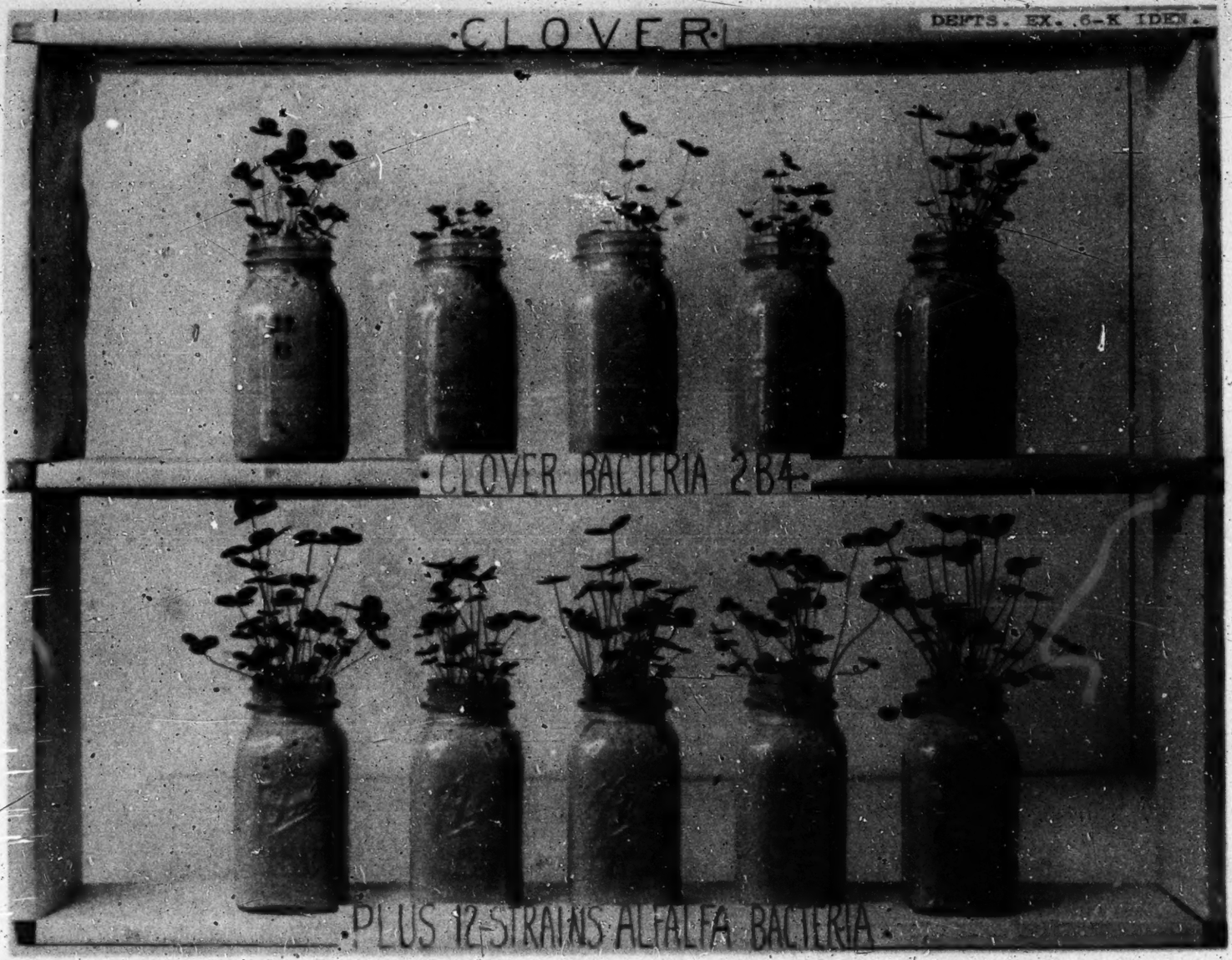
461-462

DEPTD, EX. 6-I IDEN.

463-464

OVER
DEPTS. EX. 64J IDEN.
CLOVER BACTERIA 2B3
PLUS 12-STRAINS ALFALFA BACTERIA

465-466


CLOVER
DEPTS. EX. 6-K IDEN.
CLOVER BACTERIA 2B4
PLUS 12 STRAINS ALFALFA BACTERIA

467-468

CLOVER
DEPTS. EX. 6-L IDEN.
12 STRAINS CLOVER BACTERIA
PLUS 12 STRAINS ALFALFA BACTERIA
469-470

906 DEFENDANT'S EXHIBIT 7.

The Effect of Alfalfa Nodule Bacteria (Rh. meliloti) on the Activity of Clover Nodule Bacteria (Rh. trifolii).

I. H. Curie

The result of these tests demonstrate to a scientist that there is no antagonism or inhibitive effect growing out of a combination of different groups of Rhizobium bacteria.

On January 27, 1944, a carefully controlled series of greenhouse tests was set up to check this theory of inhibition between the bacteria of the alfalfa and clover groups.

The tests were made by planting red clover seeds in quart Mason jars containing sterile quartz sand supplemented with all essential mineral nutrients except nitrogen and were inoculated as indicated below. Each group or series of seeds was planted in a series of six jars. Six plants were allowed to grow in each jar. Sterile distilled water was added as necessary.

1. One jar of each series was uninoculated.
2. Twelve series of jars were set up and each series inoculated with one strain of clover nodule bacteria in single culture unmixed with bacteria of other species.
3. Twelve series of jars were set up and each series inoculated with one strain of clover nodule bacteria mixed with a suspension of alfalfa nodule bacteria containing the same strains as used in the preparation of Legume-Aid.

The plants were allowed to grow in the greenhouse under identical conditions for a period of two months. At the end of this period the plants in the uninoculated jars were dead or dying and so small that no top growth could be harvested. The plants in the inoculated jars were cut at the surface of the sand, dried to constant weight in the oven and the weight for each jar recorded. The results are summarized below:

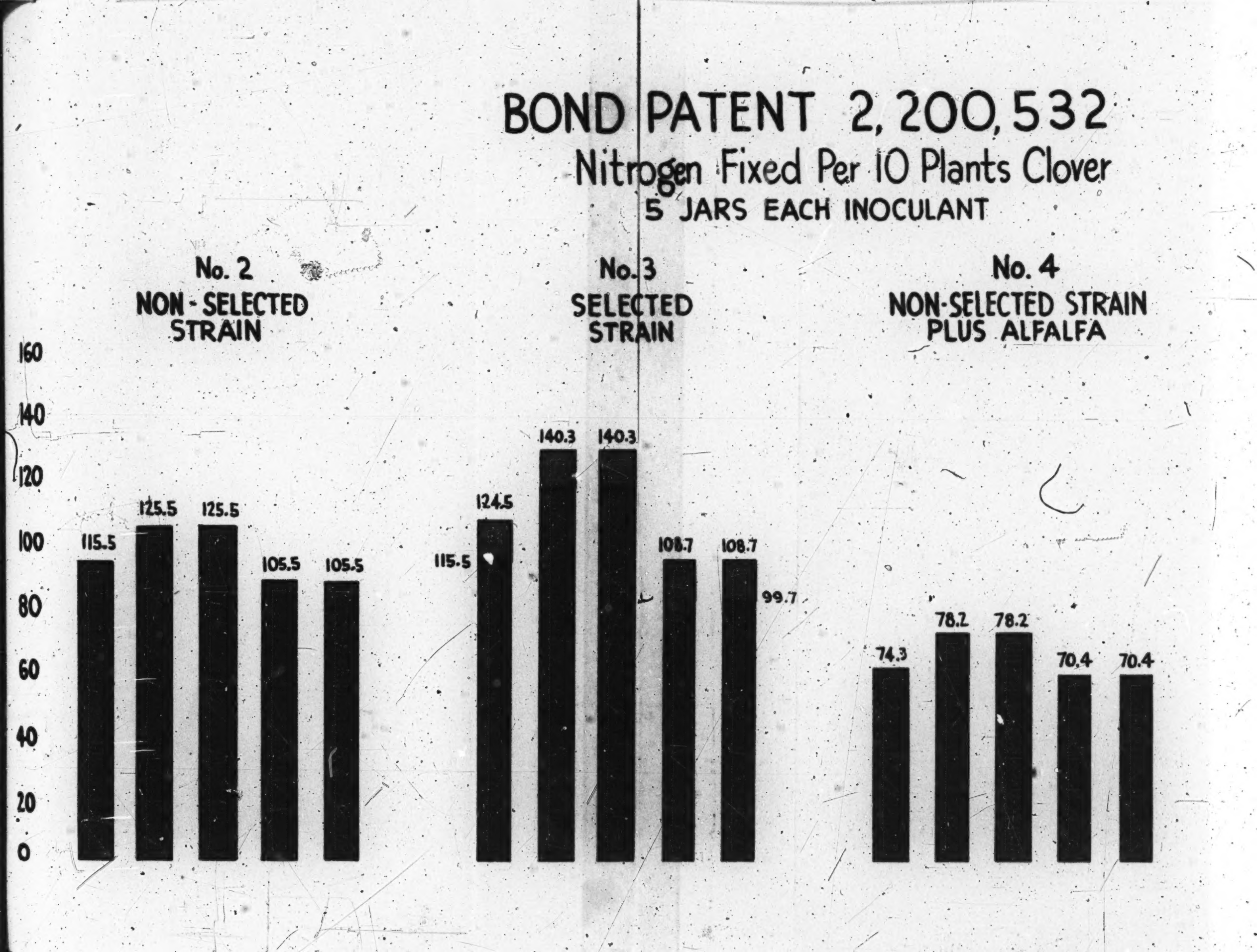
Clover Strain Number	Isolated (Year)	Plant Weight Per Jar	Effect of Alfalfa
201 Clover alone	1922	242 mg.	+158 mg. *
201 plus Alfalfa		420	
202 Clover alone	1935	1226	—116 *
202 plus Alfalfa		1110	
2A1 Clover alone	1932	922	+ 98
2A1 plus Alfalfa		1020	
2A2 Clover alone	1932	1198	+ 82 *
2A2 plus Alfalfa		1280	
2A3 Clover alone	1921	1212	— 62
2A3 plus Alfalfa		1150	
2A4 Clover alone	1941	1162	+152 *
2A4 plus Alfalfa		1314	
2A5 Clover alone	1941	1248	+ 98
2A5 plus Alfalfa		1346	
2A6 Clover alone	1941	1120	+ 20
2A6 plus Alfalfa		1140	
907 2B1 Clover alone	1920	1172	— 20
2B1 plus Alfalfa		1152	
2B2 Clover alone	1926	1146	— 20
2B2 plus Alfalfa		1126	
2B3 Clover alone	1938	1088	+ 62 *
2B3 plus Alfalfa		1150	
2B4 Clover alone	1938	780	— 8
2B4 plus Alfalfa		772	
AVERAGES			
Clover alone		1043	+ 39
plus Alfalfa		1082	

* Difference statistically significant.

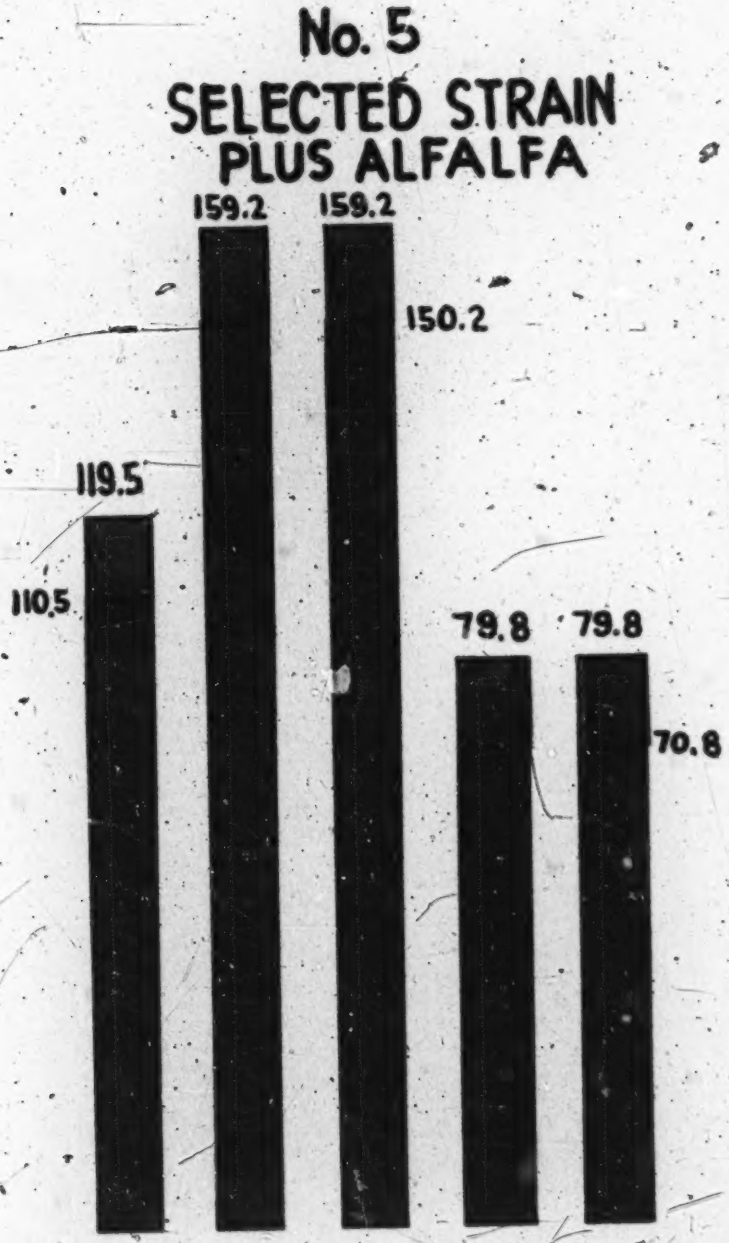
BOND PATENT 2,200,532

Nitrogen Fixed Per 10 Plants Clover

5 JARS EACH INOCULANT



DEFTS. EX. 8 IDEN.



909 DEFENDANT'S EXHIBIT 9.

June 30
1941

Dr. H. W. Bachelor
Ohio Agri. Expt. Station
Wooster, Ohio
Dear Dr. Bachelor:

Enclosed you will find the announcement of our Combination Culture for alfalfa and clover.

This past season we produced a quantity of this mixed culture under regular production methods, not laboratory experimental methods. This goods was date coded and sent into one section of the country in place of our regular Alfalfa and Clover. Six samples were picked up and tested by the state laboratory and all were found satisfactory. We are not using "alpha strains."

I believe Mr. Bond has written you that he has resigned from our organization and gone back to Kalo Inoculants. We were amazed, but I am sure we will be able to find a satisfactory man in the near future.

With best regards, I am,

Yours very truly,

I. H. Curie

IHC:HE

ELECTROS FOR YOUR CATALOG

If you do not want to use the full page space or the half page space, you can use smaller space and some of the electros listed below. **US TO GET UP THE COPY FOR YOU**, telling us just what size space you have. We will do this promptly without charge.

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Cut No. 24

Cut No. 30

Cut No. 29

Cut No. 10

Cut No. 7

Cut No. 31

Cut No. 11

Cut No. 14

Cut No. 42

Cut No. 13

Cut No. 12

Cut No. 07

Cut No. 06

476-477

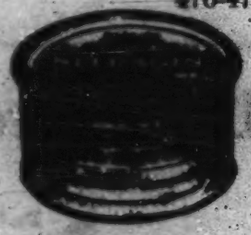
Awarded Gold Medal World's Fair
St. Louis, 1904—10 Years Ago

The Improved Soil Inoculator, Nobbe-Hiltner Process

NITRAGIN

Restores and Maintains Soil Fertility

THE ORIGINAL SOIL INOCULATOR



Sold in the Greatest Problem
An Exclusive Feature

NITRAGIN FACTS

THAT BEAR GREAT WEIGHT

1. IT is the first of its kind, the original pure culture legume inoculator; to which facts scientific text books and trademark records bear indisputable evidence. (Consult any Library.)

2. IT was invented by Nobbe & Hiltner, world famous soil experts, who discovered the science of legume inoculation with nitrogen fixing bacteria in 1896—TWENTY-SEVEN YEARS AGO.

3. IT was registered in the United States with trademarks "NITRAGIN" December 6th, 1898—TWENTY-FIVE YEARS AGO.

4. It was awarded a Gold Medal at the St. Louis World's Fair 1904—NINETEEN YEARS AGO.

5. IT has stood up under every test for TWENTY-SIX YEARS.

6. IT has been marketed in practically every agricultural country on earth for more than TWENTY YEARS.

7. IT has thrived and grown stronger in popular opinion year after year, in spite of hardships, prejudices and the rigid tests to which every great invention is more or less subjected.

8. IT is one of the most universally and highly endorsed products on the world's markets. IT HAS MANY IMITATORS.

9. IT is endorsed by Farmers, Experimental Stations, Agricultural Colleges, Agricultural Journals, Text Books, Soil Experts, Scientists and Educators the world over.

To see that your high grade seed is successfully inoculated means more than just a side-line profit. It means MORE SEED SALES, MORE SEED PROFITS, AND MORE SATISFIED CUSTOMERS.

CATALOGUE NITRAGIN

SOLD ON BUSHEL BASIS—NOT ACRE SIZES

Prominent Seed Houses heartily endorse this plan of selling NITRAGIN on the bushel basis instead of in acre sizes. This plan is practical. We predict all cultures for soil inoculation will soon be sold on the bushel basis.

A Great Price Reduction

Instead of the farmer paying one dollar per acre or more, he will only pay one dollar per bushel of seed inoculated. This cuts the price to the farmer to as low as 20c per acre, depending on the amount of seed he sows per acre.

A Double Profit to Dealers

As you will see by looking at the dealers prices on the inside page, your profits from the sale of NITRAGIN are BIG. We are told by our seed house customers that their profit on seed is also increased—because NITRAGIN used with their legume seed ASSURES A BETTER CROP.

LET NITRAGIN be a feature this year—it's too big for a side line

Def. [redacted] EX. 10 IDEN.

(FOR FULL PAGE) No. 60

Larger Crops in Field and Garden

If you use

The Improved Soil Inoculator, Nitro-Culture Process

NITRAGIN

Restores and Maintains Soil Fertility

Registered in the United States Dec. 6th, 1898.
25 YEARS AGO.

Awarded Gold Medal
World's Fair - St. Louis
1904.
19 YEARS AGO.

Alfalfa, clovers, soybeans, vetches, peas, beans, cowpeas, peanuts and other legumes should always be inoculated with Nitragin. The lack of necessary bacteria in the soil is often the cause of a poor legume crop—a weak start—or a total failure.

The modern farmer says: "I inoculate with Nitragin because I want a better "catch." I want plenty of those little nodules or germ lumps on every root. Nitragin nodules are plant food (nitrogen from the air) They mean well fed legume plants; big crop; less winter killing; better soil."

A Great Price Reduction

NITRAGIN Costs Less

NITRAGIN now comes in bushel sizes instead of acre sizes. This means that it costs you less per acre than inferior imitations. If you sow 12 pounds of seed to the acre it will only cost you 20 cents for your Nitragin. It means that

Your Dollar Goes Further With NITRAGIN

Nitragin is an old, reliable inoculator. At the low price of 20 to 33 cents per acre you cannot afford to sow legumes without it. Nitragin is put up in the modern ventilated package; in a rich, soil-like packing medium. The contents are always fresh, living bacteria to feed your plants. Easy to use.

EVERY GARDEN NEEDS NITRAGIN

New Garden Culture For Peas and Beans
See Below

PRICES

½ Bushel size.....40c Postage.....4c
¼ Bushel size.....60c Postage.....5c
1 Bushel size.....$1.00 Postage.....7c
5 Bushel size.....$4.75 Postage.....15c
Garden (peas, beans, sweet peas - three in one package).....20c Postage.....2c

STATE WHAT CROP YOU WANT THE NITRAGIN FOR

Your Best Paying

Will be one with—The Improved Soil Inoculator, Nitro-Culture Process NITRAGIN Restores and Maintains Soil Fertility —With

Use Your Catalog for NITRAGIN

You give counter and catalog space for selling seed. For your c
should **use catalog space for NITRAGIN.**

(FOR HALF PAGE) (No. 61)

IMPORTANT

NITRAGIN COSTS YOUR FARMER LESS per acre—make this your big point when pushing Nitragin sales. Your profit per can is as big as ever. This is because we are now selling Nitragin on a bushel basis instead of the acre size.

Neatly designed carton containing 12 Garden Size for counter display.
A neat cut for Garden Catalogues.

LARGER CROPS in FIELD a

If you use

Registered in the United States Dec. 6th, 1898.
25 YEARS AGO.

NITRAGIN Restores and Maintains Soil Fertility

Alfalfa, clovers, soybeans, vetches, peas, beans, cowpeas, peanuts and other le... Nitragin. The lack of necessary bacteria in the soil is often the cause of a po... failure.

A Great Price Red

NITRAGIN Costs Less

Nitragin now comes in bushel sizes instead ... bushel size per acre than inferior imitations. If ... 12 pounds of seed to the acre it will only cost you 20 cents for your Nitragin. It ...

Your Dollar Goes Furthe

Nitragin is an old, reliable inoculator. At ... price of 20 to 33 cents per acre you cannot afford to sow legumes without it. Ni... put up in the modern ventilated package; in a rich, soil-like packing medium. ... contents are always fresh, living bacteria to feed your plants. Easy to use.

EVERY GARDEN NE

New Garden Culture for ...
See Below

PRICES

½ Bushel size.....40c P...
¼ Bushel size.....60c P...
1 Bushel size.....$1.00 P...
5 Bushel size.....$4.75 P...
Garden (peas, beans, sweet peas - three in one package).....20c P...

STATE WHAT CROP YOU WANT THE NITRAGIN F...

OUR 1923 PRICES

Small ... are sent direct to wholesale and catalogue seed houses, or direct from our laboratory to the customers of ... seed houses. Prices are:

			Wholesale	Retail	Consumer	
½ Bu.	Inoculates	12 lb of seed	$ .20	$ .30	$ .40	F. O. B. Milwaukee
¼ "	"	30 " "	.30	.45	.60	
1 "	"	60 " "	.50	.70	1.00	
5 "	"	5 Bu. "	3.45	3.25	4.75	

QUANTITY PRICES—NON-RETURNABLE

10 Tins — based on BUSHEL size — Discount 10 %
100 " " " " " 15 % F. O. B.
250 " " " " " 20 % Milwaukee

You can order all one kind or assorted

EXPLANATION:
4—¼ Bu. are equivalent to ONE bushel
2—½ " "
1—5 Bu is equivalent to FIVE bushels

GARDEN SIZE

For Peas, Sweet Peas and Beans (3 in one). Sent in neatly assorted cartons for Counter display. 1 dozen in each carton.

	Wholesale	Retail	Consumer	
Each Lone $	1.20—12c each	$ 1.44—12c each	20c Ea.	F. O. B. Milwaukee
12 Cartons	12.00—1c each	14.40—12c each	20c Ea.	

The NITRAGIN Company, Inc. 457-9 E. Water St. Milwaukee, Wis.

r Best Paying Page

e with- NITRAGIN -With 100% Profit

Restores and Maintains Soil Fertility

Use Your Catalog for NITRAGIN:

unter and catalog space for selling seed. For your own profit you
catalog space for NITRAGIN.

OUR HALF PAGE: (No. 10)

r

FARMER
r big point
Your profit
is because
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logues.

LARGER CROPS in FIELD and GARDEN

If you use

NITRAGIN

Restores and Maintains Soil Fertility

Registered in the United States Dec. 6th, 1898. 25 YEARS AGO.

Awarded Gold Medal World's Fair - St. Louis 1904. 19 YEARS AGO.

Alfalfa, clovers, soybeans, cowpeas, beans, peanuts and other legumes should always be inoculated with Nitragin. The use of necessary bacteria, in the soil is often the cause of a poor legume crop. A good inoculation is also

A Great Price Reduction

NITRAGIN Costs Less

Nitragin now comes in Bushel sizes instead of acre sizes. This means that it only costs one cent per bushel for inoculation. If you sow 1 pound to the acre it will cost you 10 cents for your Nitragin. It means this:

Your Dollar Goes Further With NITRAGIN

Nitragin is an old, reliable inoculation. At the low rates of 40 to 60 cents per acre you cannot afford to sow legumes without it. Nitragin is sold by all the leading seedsmen, in a form suitable to your soil. Ask your dealer for Nitragin. Refuse to buy other stocks. Keep to us.

EVERY GARDEN NEEDS NITRAGIN

New Garden Culture for Peas and Beans
See Below

PRICES

½ Bushel size	40c	Postage 5c
¼ Bushel size	25c	Postage 3c
1 Bushel size	$1.75	Postage 7c
5 Bushel size	$7.75	Postage 40c
Garden (peas, beans, sweet corn - One to new package)	25c	Postage 3c

STATE WHAT CROP YOU WANT THE NITRAGIN FOR

PRICES

GARDEN SIZE

For Peas, Sweet Peas and Beans (5 in each). Sent in neatly decorated cartons for Garden Packet. 1 pound in each carton.

Wholesale	Retail	Consumer
[illegible]	[illegible]	[illegible]

F. O. B. Milwaukee

Milwaukee, Wis.

DIMENSIONS OF ADS

These full page and half page forms are electros. Order them by number. They are [illegible] Inches. We have these [illegible] special ruled pages:

No. 10	Full page—4½ in. wide by 6½ in. deep
No. 20	Full page—4½ in. wide by 3 in. deep
No. 30	Full page—7 in. wide by 3 in. deep
No. 40	Half page—4½ in. wide by 3½ in. deep
No. 50	Half page—4½ in. wide by 3 in. deep
No. 60	Half page—3½ in. wide by 4½ in. deep
No. 70	Half page—3 in. wide by 4 in. deep
No. 80	Half page—4½ in. wide by 2½ in. deep

478-479

DEFTS. EX. 11-A IDEN.

DEFTS. EX. 11-B IDEN.

DEFTS. EX. 11-C IDEN.

DEFTS. EX. 11-D IDEN.

DOES ONE SPECIES OF LEGUME BACTERIA PRODUCE AN INHIBITORY EFFECT 482-483
ON ANOTHER SPECIES WITHIN THE SAME CULTURE?

In August, 1944, a greenhouse experiment was planned with several objects in view: (1) to test the nitrogen-fixing efficiency of five strains of alfalfa bacteria, Rhizobium meliloti, on alfalfa plants, and (2) to determine if there exists any antagonism or possible inhibition of nitrogen fixation between Rhizobium meliloti and Rhizobium trifolii when grown in the same culture.

The history of the alfalfa strains used in this study follows:

Strain No.	Source of Strain	Date Obtained
3DOa1	Commercial Farmogerm Culture	1912
3DOa7	Alfalfa Nodule Caledonia, Minnesota	1932
3DOa10	Nitragin Collection Formerly No. 103	1926
3DOa12	U. S. D. A. Strain No. 436	1928
3DOa15	O. A. C. Alfalfa Strain Dr. Halverson	1928

The history of the red clover strains used in this study follows:

Strain No.	Source of Strain	Date Obtained
3DOk1	Red Clover Nodule Near Sterling, Ill.	1934
3DOk4	Red Clover Nodule Caledonia, Minnesota	1932
3DOk5	Red Clover Nodule, Illinois	193 4
3DOk7	Red Clover Nodule, Michigan	1934
3DOk13	Red Clover Nodule, Wisconsin	1935

EXPERIMENTAL

A pure culture of each single strain was inoculated into 250 c.c. of yeast-mannitol liquid No. 79 on September 1, 1944. On September 8, 1944, single strain humus cultures were prepared by mixing 40 c.c. of each liquid suspension of bacteria with 50 gms. 200 mesh dried and partially sterilized humus and 12.5 gms. finely ground limestone to neutralize the acidity.

Mixed cultures were prepared by mixing 20 c.c. of a single strain alfalfa liquid suspension plus 20 c.c. of a composite liquid suspension of red clover bacteria with 50 gms. of humus and 12.5 gms. of limestone. This composite liquid suspension of red clover bacteria was prepared by mixing together 50 c.c. of liquid from each of the five pure culture suspensions of red clover bacteria, making a total of 250 c.c. of this composite liquid.

The culture treatments used in this experiment were as follows:

No. 1 Single strain alfalfa No. 3DOa1
No. 2 Single strain alfalfa No. 3DOa1 plus red clover bacteria
No. 3 Single strain alfalfa No. 3DOa7
No. 4 Single strain alfalfa No. 3DOa7 plus red clover bacteria

DEPTS. EX. 13 IDEN.

Defendants
Exhibit No. 13
T. B. D.

No. 5 Uninoculated controls
No. 6 Composite mixture of all five single strain alfalfa bacteria
No. 7 Composite mixture of all five single strain alfalfa bacteria plus composite mixture of five single strains of red clover bacteria
No. 8 Single strain alfalfa No. 3D0a10
No. 9 Single strain alfalfa No. 3D0a10 plus red clover bacteria
No. 10 Single strain alfalfa No. 3D0a12
No. 11 Single strain alfalfa No. 3D0a12 plus red clover bacteria
No. 13 Single strain alfalfa No. 3D0a15
No. 14 Single strain alfalfa No. 3D0a15 plus red clover bacteria

One-half gallon earthenware glazed pots were prepared as shown in Figure 1. A layer of coarse gravel was first added, the watering tube put in place, washed bank sand was added next and the sand was thoroughly moistened with Bryan's modified Crone's solution. Each pot was covered with wrapping paper tied and sterilized in the autoclave for five hours at 15 lbs. pressure.

The alfalfa seeds were first freed of legume bacteria and a sufficient number inoculated with each culture treatment to plant five pots. Planting of the alfalfa was done on September 25. After planting a thin layer of fine gravel was put on top of the sand in each jar to reduce chances for contamination. Extreme care was exercised in watering; sterilized distilled water was added when needed and at 30-day intervals more sterilized Crone's solution was added.

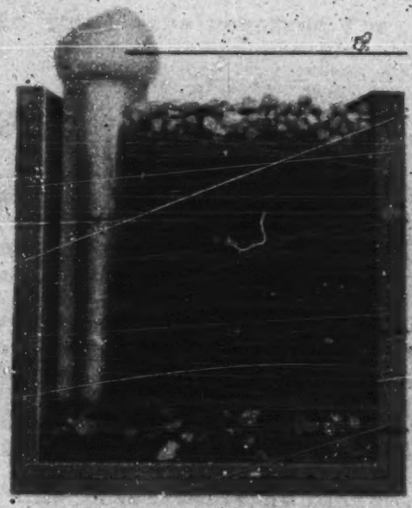

Figure 1

The plants were photographed on January 9, 1945, and harvested on January 18, 1945. The roots were washed free of sand and the whole plants from each pot were dried, weighed, ground, and samples analyzed for total nitrogen by the standard Kjeldahl method.

RESULTS

A complete set of pictures obtained in this experiment is attached hereto. A careful study of each picture would tend to disprove the statement that "it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen-fixation by the plant and the organism was inhibited or even prevented."

Since one of the main objects of this experiment was to determine any possible inhibitive effect of the red clover bacteria on the nitrogen-fixing ability of the alfalfa strains, the data obtained from the total nitrogen determinations were subjected to a critical statistical analysis.

The results of this analysis are as follows:

		3D0a1	3D0a7	3D0a10	3D0a12	3D0a15	Mixture 5Strains	Total
Alfalfa	ΣX	108.2	123.0	145.8	135.3	133.1	142.2	787.6
	ΣX^2	2,476.96	3,088.06	4,355.02	3,756.73	3,593.29	4,236.7	
Alfalfa plus Red Clover	ΣX	132.1	161.3	144.9	152.2	151.0	177.0	898.5
	ΣX^2	3,525.81	5,556.85	4,223.89	4,787.70	3,488.92	6,329.52	
Total		240.3	284.3	290.7	287.5	284.1	319.2	1,686.1
Difference		23.9	38.3	.7	16.9	2.1	34.9	5%= 33.5

A difference of 33.5 between strain totals are required for statistical significance.

CONCLUSIONS

The conclusions, which may be drawn from these data, are:

(1) An over-all comparison between strains used singly in mixtures of alfalfa strains and alfalfa strains plus red clover strains is significant in favor of the alfalfa strains plus red clover strains mixture.

(2) Alfalfa strain No. 3D0a7 at the 5% level is significantly better when mixed with red clover strains that when used singly. Mixtures of five strains of alfalfa bacteria plus five strains of red clover bacteria are significantly better than the five strains of alfalfa alone.

(3) Alfalfa strains Nos. 3D0a1 and 3D0a12 show an apparent beneficial result.

(4) Alfalfa strains Nos. 3D010 and 3D0a15 show practically no difference between the use of the composite and the single culture.

(5) These data strongly support the fact that strains from different cross-inoculation groups--namely, the alfalfa and clover groups--can be mixed and grown together and that no inhibitive effect is demonstrated when they are mixed.

(6) The set of pictures offer strong evidence to support conclusion No. 5.

Lewis W. Erdman
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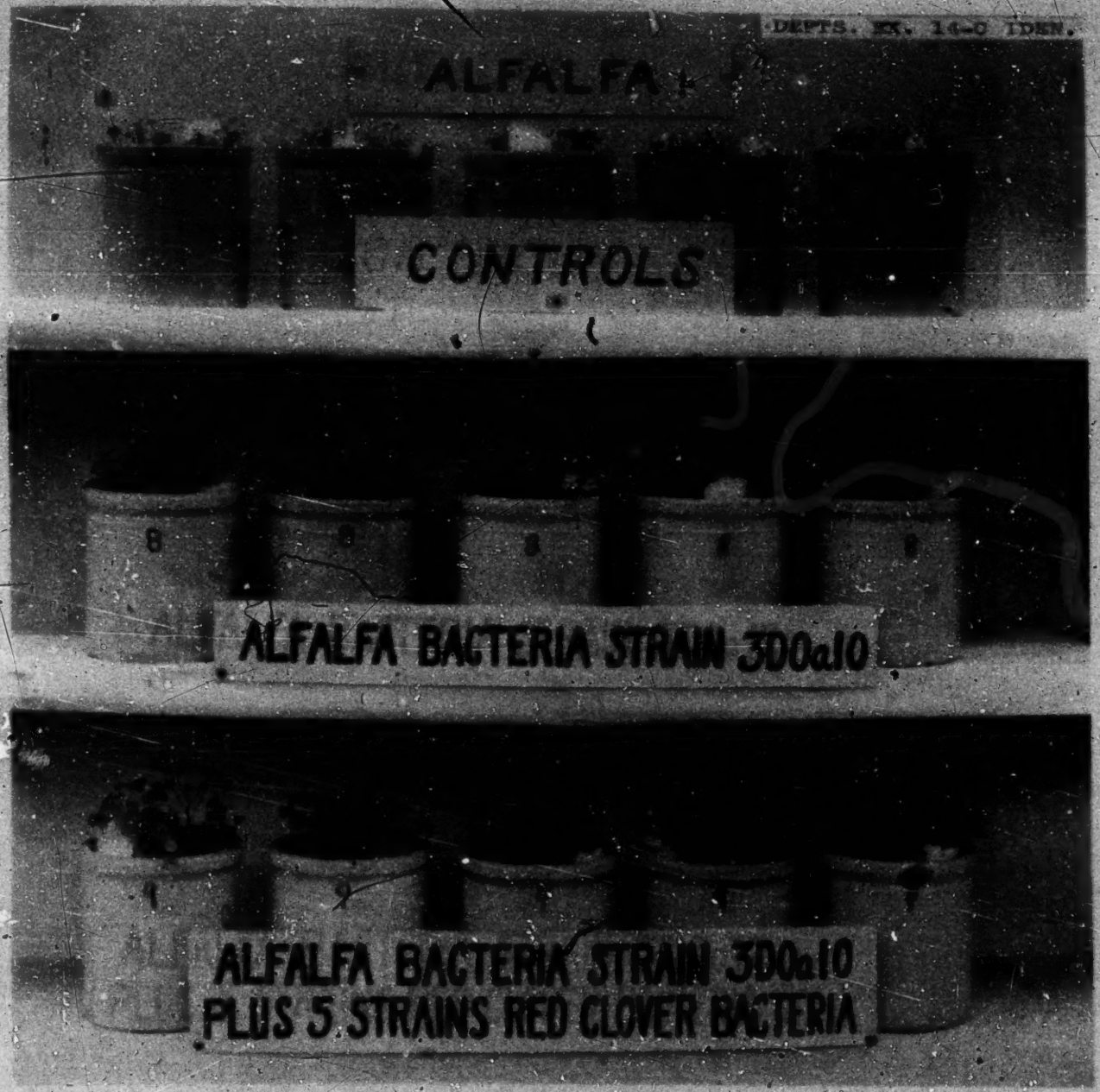
DEFTS. EX. 13 IDEN.

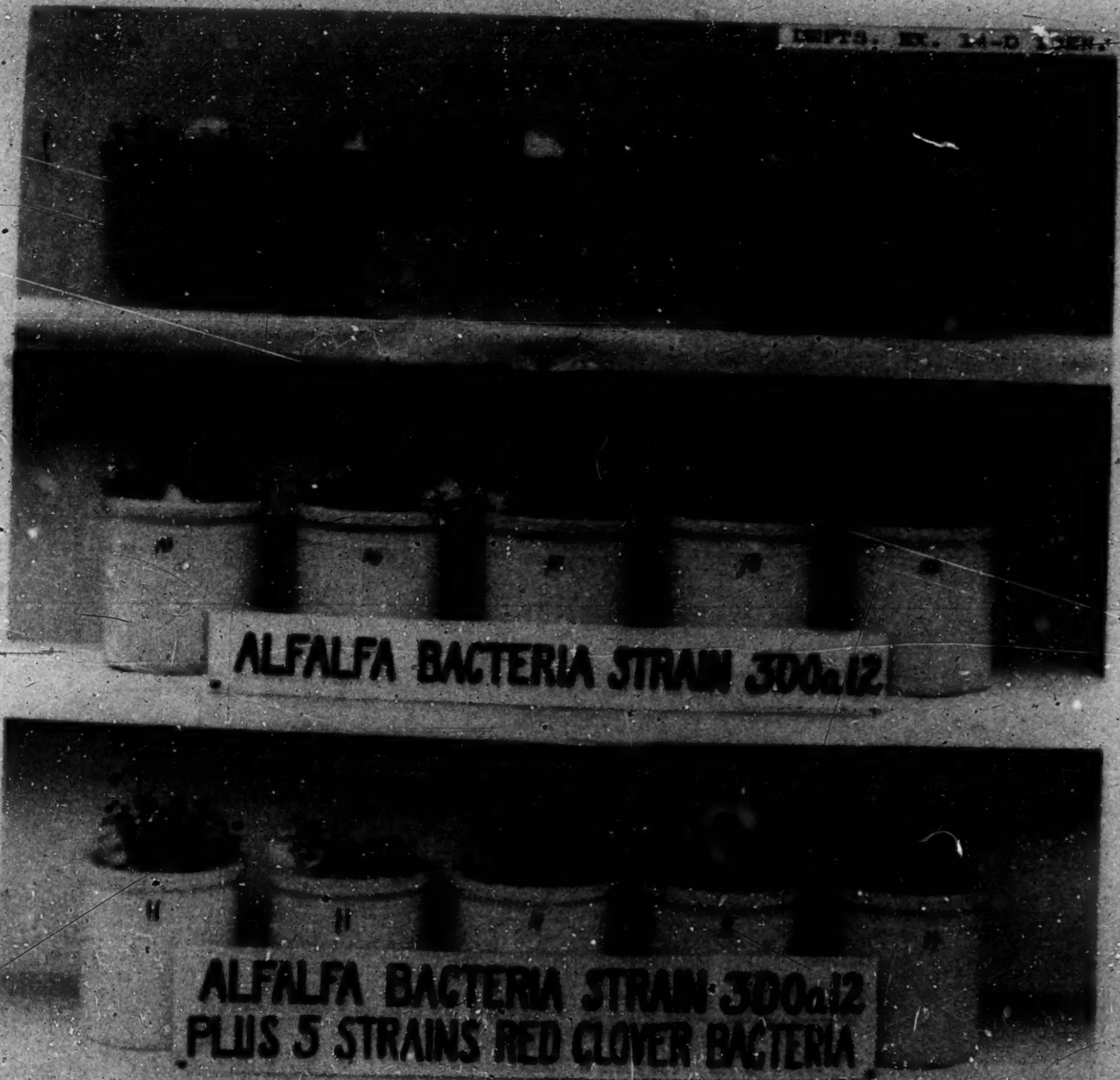
DEPTS. EX. 14-A IDEN.

488-489

ALFALFA
CONTROLS
ALFALFA BACTERIA STRAIN 3D0a7
ALFALFA BACTERIA STRAIN 3D0a7
PLUS 5 STRAINS RED CLOVER BACTERIA

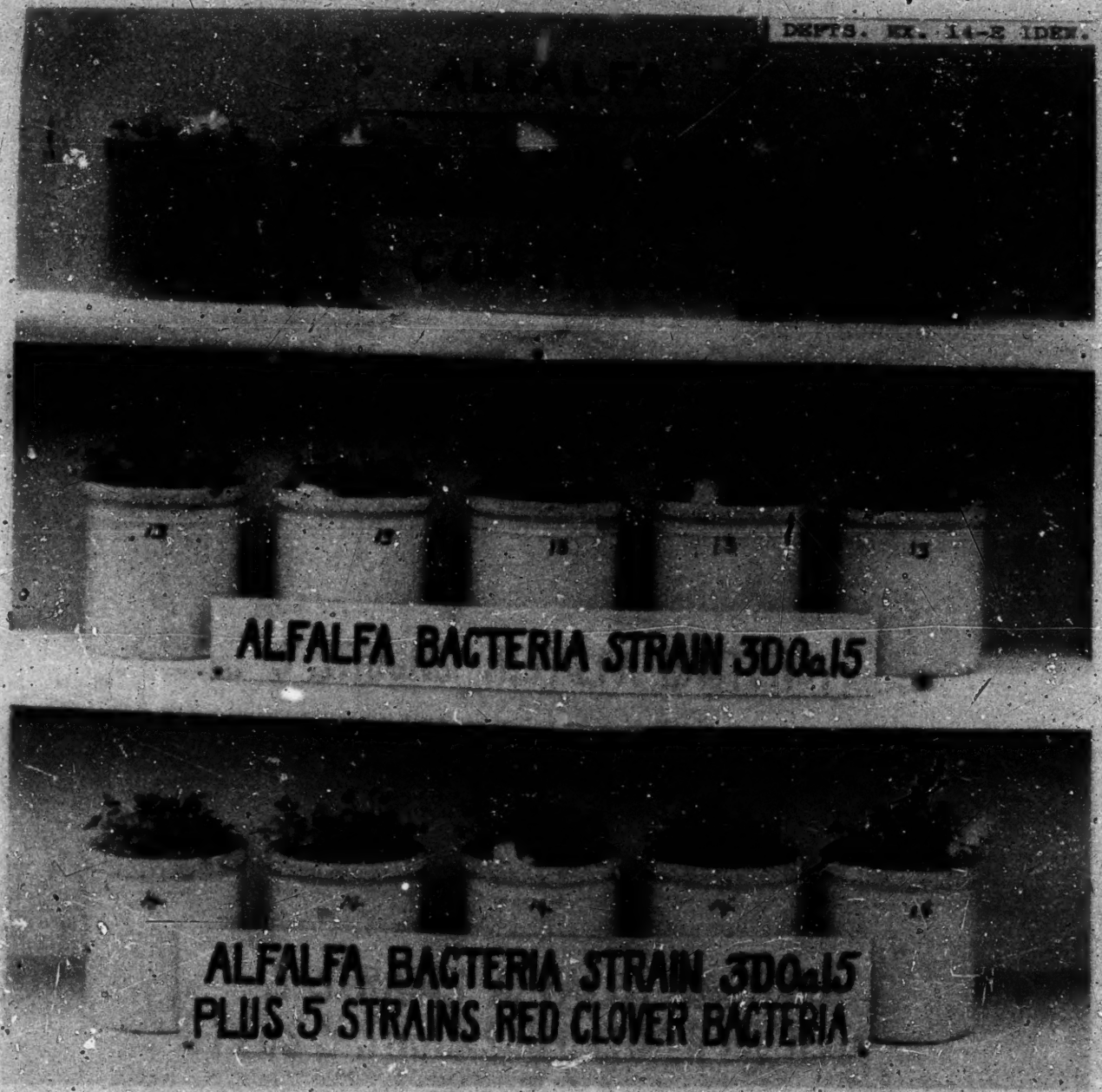

DEPTS. EX. 14-C IDEN.
ALFALFA
CONTROLS
ALFALFA BACTERIA STRAIN 3D0a10
ALFALFA BACTERIA STRAIN 3D0a10
PLUS 5 STRAINS RED CLOVER BACTERIA
492-403

DEPTS. EX. 14-D 1 DEN.



494-495

DEPTS. EX. 14-2 IDEN.



496-497

ALFALFA
CONTROLS
5 STRAINS ALFALFA BACTERIA
5 STRAINS ALFALFA BACTERIA
PLUS 5 STRAINS RED CLOVER BACTERIA

498-490

927

DEFENDANT'S EXHIBIT 15-A.

EXPERIMENT NO. 1—CRIMSON CLOVER

Sept. 22 to Jan. 7, 1938

Culture No.	Dry Weight grams	Percentage Nitrogen	Total Nitrogen
3DOQ1(a)	9.25	1.24	115.6*
3DOQ2(a)	6.25	1.18	74.0
3DOQ2(c)	8.00	1.28	102.5
3DOQ2(d)	7.50	1.32	99.0
3DOQ2(e)	8.00	1.63	130.9
Control	1.25	.94	1.2

* Each datum based on 20 plants

928

CRIMSON CLOVER—Trifolium incarnatum

Oct. 19, 1939 to Jan. 20, 1940

Strain	Dry Weight 36 pl. gms.	Percentage Nitrogen	Total Nitrogen mgms.
3DO11	3.54	1.80	63.6
3DOY1	4.41	2.08	93.9
3DOM5	1.61	1.68	27.9
3DOK4	3.09	1.80	55.5
3DOK7	1.39	1.40	19.5
3DOK5(a)	4.58	2.08	95.5
3DOK13	1.45	1.40	20.0
3DOK21	3.15	1.60	50.5
3DOK23	1.93	1.16	22.4
3DOK24	1.76	1.14	20.0
3DOQ1(a)	4.20	1.92	80.6
3DOQ2(c)	3.60	1.96	70.7
Control	1.14	1.24	14.1
Control	1.27	1.28	16.3

Strain	Host-plant	Source	Date
3DO11	Alsike clover		
3DOY1	McNeill Clover		
3DOM5	White clover		
3DOK4	Red clover		
3DOK7	" "		
3DOK5(a)	" "		
3DOK21	" "		
3DOK23	" "		
3DOK24	" "		
3DOQ1(a)	Crimson clover		
3DOQ2(c)	Crimson clover		

929 DEFENDANT'S EXHIBIT 15-B.

RED CLOVER—Trifolium pratense

Oct. 18, 1939 to Jan. 15, 1940

Strain	Dry Weight 36 pl. gms.	Percentage Nitrogen	Total Nitrogen mgms.
1DO11	2.76	1.82	50.0
1DOY1	.84	.75	11.1
1DOM5	2.94	1.82	54.9
1DOK4	2.50	1.88	66.0
1DOK7	2.54	1.74	61.5
1DOK5(a)	2.90	1.02	79.0
1DOK13	1.23	1.40	17.9
1DOK31	3.59	1.80	64.5
1DOK29	4.14	1.80	74.5
1DOK24	1.07	1.20	12.3
1DOQ1(a)	3.86	1.90	69.5
1DOQ2(c)	3.96	1.92	76.0
Control	.97	.76	11.5
Control	1.04	1.40	14.8
Control	1.07	1.02	10.9

Strain	Host-plant
1DO11	Alsike clover
1DOY1	McNeill clover
1DOM5	White clover
1DOK4	Red clover
1DOK7	" "
1DOK5(a)	" "
1DOK13	" "
1DOK31	" "
1DOK29	" "
1DOK24	" "
1DOQ1(a)	Crimson clover
1DOQ2(c)	Crimson clover

930 DEF. EX. 15-B.

EXPERIMENT NO. 1—RED CLOVER

Sept. 21 to Dec. 14, 1937

Culture No.	Dry Weight grams	Percentage Nitrogen	Total Nitrogen
3DOK1(a)	5.0	2.62	131.0*
3DOK2	2.0	2.92	58.4
3DOK3(37a)	2.5	3.18	79.5
3DOK5(a)	4.0	2.64	106.7
3DOK6	2.0	3.08	61.5
3DOK7	5.5	2.76	151.8
3DOK8	4.0	2.84	114.0
3DOK12	4.5	2.74	123.8
3DOK13	5.0	3.20	160.0
3DOK14	4.0	2.80	112.0
3DOK15	.3	1.36	4.0
3DOK16	1.2	2.50	30.0
3DOK19	2.5	2.80	70.0
3DOK18	3.0	2.94	88.0
3DOK20	.2	1.21	2.6
Control	.2	1.18	2.4
Control	.2	1.27	2.5

* Each datum based on 10 plants.

931 DEFENDANT'S EXHIBIT 15-C.

TABLE 3

NITROGEN FIXATION BY TWENTY STRAINS OF R. MELILOTI ON ALFALFA, BURR CLOVER AND SWEET CLOVER

Oct. 15 to Dec. 10, 1926

	Alfalfa		Burr Clover		Sweet Clover	
Strain	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen
3DOa1	1.6	49.0	.3	5.0	2.5	82.5
	1.6	44.0	.4	4.2	2.6	83.6
	1.3	30.0	.4	4.5	2.6	79.4
	4.5	114.0	1.1	13.8	7.7	245.6
3DOa7	1.1	27.5	.3	3.1	2.3	68.5
	2.7	67.5	.3	4.1	2.7	95.0
	2.7	74.0	.3	3.7	2.9	100.0
	6.5	169.0	.7	8.9	8.3	293.5
3DOa10	2.3	62.0	.4	3.1	3.1	109.0
	3.0	81.0	.3	4.3	3.4	114.0
	2.5	75.0	.3	3.8	3.3	111.0
	7.7	219.0	1.0	11.2	9.3	334.0
3DOa12	2.4	67.0	.4	3.8	3.5	107.0
	3.4	89.1	.4	5.0	3.5	125.0
	3.1	80.4	.3	3.5	2.9	99.8
	8.9	236.5	1.0	12.3	9.9	331.6
3DOa14	2.3	64.1	.3	4.0	2.9	95.0
	2.6	71.0	.3	3.3	3.0	93.4
	2.3	63.5	.3	4.1	2.6	88.5
	7.3	198.6	.8	12.0	8.5	275.9
3DOa15	2.8	80.5	.3	3.3	1.3	43.0
	2.6	74.0	.3	4.0	.8	27.0
	2.5	74.0	.3	3.6	1.6	48.0
	7.9	228.5	.8	11.4	3.8	128.0
3DOa17	2.0	56.5	.3	3.9	2.1	68.0
	1.9	55.5	.4	3.7	1.7	39.0
	2.0	60.0	.3	4.6	1.8	41.0
	5.9	172.0	.9	12.5	5.4	186.0
3DOa26	.3	3.3	.3	3.4	.3	3.3
	.5	4.1	.5	4.0	.2	2.4
	.5	3.9	.3	5.2	.4	5.1
	1.3	11.3	.8	12.5	.9	11.3
3DOa1(a)	2.3	65.0	1.7	47.3	.8	9.4
	2.6	69.0	1.8	46.5	1.1	12.3
	1.9	59.3	1.8	51.8	.8	10.9
	6.8	193.3	5.3	145.0	2.5	32.6
3DOa1(d)	1.9	47.7	1.1	31.4	2.5	93.3
	2.4	67.0	1.2	35.9	2.6	75.0
	2.0	53.5	1.1	30.2	2.4	75.0
	6.3	167.3	3.5	97.5	7.5	223.5
3DOC1	2.1	57.0	.3	3.4	.3	17.7
	1.8	49.7	.3	4.1	1.1	30.1
	2.6	64.0	.3	5.0	1.0	18.3
	6.5	170.7	.8	12.5	3.0	66.1

932 TABLE 3 (Continued)

Strain	Alfalfa		Burr Clover		Sweet Clover	
	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen
	1.3	18.7	2.5	48.5	1.0	22.5
3DOC2	1.5	12.2	1.7	30.5	1.4	29.0
	1.3	13.6	1.7	40.3	1.2	31.0
	4.0	44.3	5.7	149.1	3.6	82.5
	1.0	20.0	.9	21.0	1.7	37.5
3DOC3	.8	17.0	1.1	37.6	1.9	52.0
	1.1	20.4	.8	18.9	1.3	29.0
	2.9	57.4	2.8	67.5	6.4	196.0
	1.5	37.0	1.4	43.5	2.3	54.5
3DOd2	1.9	43.2	1.4	40.5	3.1	78.0
	1.6	32.0	1.8	45.7	2.3	51.0
	5.0	113.2	4.4	129.7	6.6	241.5
	2.4	57.0	.8	7.7	2.3	52.0
3DOb1	2.1	59.0	.4	8.7	2.5	90.0
	2.2	62.0	.3	8.5	2.0	57.0
	6.7	188.0	1.0	24.9	6.8	204.0
	2.6	57.0	.8	3.7	2.7	58.5
3DOb2	2.7	62.0	.3	4.0	3.1	88.0
	2.9	54.8	.3	5.0	2.6	73.0
	7.2	179.2	.8	12.7	8.4	262.5
	1.6	46.0	.3	2.0	2.1	59.0
3DOb7	1.3	46.0	.3	2.5	3.6	100.0
	2.2	55.0	.3	4.2	3.6	102.0
	5.6	149.0	.3	10.3	9.7	262.0
	1.3	23.2	.3	3.6	2.3	59.0
3DOb3(a)	1.7	41.5	.3	5.9	1.9	69.1
	1.2	32.5	.3	2.8	2.4	77.0
	4.3	107.2	.3	12.3	6.6	225.1
	1.1	12.1	.3	3.8	2.8	77.0
3DOb10	1.3	14.2	.3	3.1	2.1	68.0
	1.6	13.9	.3	4.7	2.6	81.0
	4.0	40.3	.7	11.4	7.3	199.0
	1.6	43.0	.4	4.3	2.4	66.0
3DOi3	1.9	61.5	.4	5.0	2.5	87.0
	1.8	51.0	.4	2.9	2.7	88.5
	5.3	155.5	1.3	13.3	7.6	257.5
	.9	4.3	.4	4.2	.4	5.3
Control	1.1	7.5	.3	3.0	.3	4.3
	.8	4.1	.3	3.4	.3	4.2
	2.8	17.9	.9	10.6	1.9	14.2
	1.3	5.9	.3	3.5	.3	3.6
Control	.9	3.1	.3	2.4	.4	4.2
	.9	3.5	.3	3.0	.4	5.4
	3.0	22.5	.8	9.9	1.0	13.2

Each datum based on 10 plants.

TABLE 8

NITROGEN FIXATION BY TWENTY STRAINS OF R. MELILOTI ON ALFALFA, BURR CLOVER AND FENUGREEK

Feb. 10 to April 17, 1939

Experiment 8

Strain	Alfalfa		Burr Clover		Fenugreek	
	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen
3DOA3	2.1	82.8	.3	3.3	.4	5.4
	2.1	84.0	.3	3.4	.4	5.4
	2.1	82.0	.4	4.3	.4	4.9
	6.3	102.5	1.0	11.1	1.3	15.7
3DOA2	1.3	82.3	.3	3.3	.4	5.4
	1.3	81.4	.3	3.0	.4	4.8
	1.3	81.9	.3	3.7	.4	4.5
	3.7	95.7	.3	9.9	1.3	14.7
3DOA7	1.3	80.4	.3	3.7	.4	4.1
	1.6	85.3	.3	4.3	.3	4.3
	1.4	82.8	.3	4.6	.3	4.8
	4.3	90.4	.3	12.1	1.0	12.9
3DOA15	1.3	81.0	.3	3.0	.3	5.3
	1.4	85.8	.4	3.4	.4	4.6
	1.5	86.9	.4	4.7	.3	3.7
	4.1	104.7	1.1	11.1	1.4	13.4
3DOA17	.7	17.3	.4	3.6	.3	4.7
	.9	22.2	.4	3.0	.4	4.4
	.9	20.6	.3	4.3	.4	5.3
	2.5	61.1	1.1	11.5	1.3	14.3
3DOA36	.3	3.6	.4	3.0	.3	3.0
	.3	4.3	.3	4.6	.3	5.3
	.3	3.7	.3	3.0	.3	3.4
	.9	11.6	.9	13.0	.9	13.6
3DOb3	1.1	24.3	.3	4.3	.4	4.3
	1.3	34.0	.3	3.0	.4	4.1
	1.3	41.0	.3	4.3	.4	4.3
	4.7	101.0	.3	12.1	1.3	13.6
3DOb7	1.7	33.0	.3	3.3	.4	4.6
	2.3	33.0	.4	3.3	.4	3.3
	2.4	34.0	.3	4.3	.4	3.6
	8.0	104.0	1.0	12.3	1.3	13.3
3DOb3(a)	1.3	40.3	.3	3.7	.3	4.6
	2.3	44.0	.3	4.3	.4	3.3
	1.3	41.3	.3	3.1	.4	5.3
	6.7	105.3	1.1	13.7	1.4	15.1
3DOb10	.7	14.0	.4	3.7	.3	4.3
	.3	16.0	.4	4.3	.3	4.3
	.3	16.3	.3	4.3	.3	4.7
	2.3	46.3	1.1	12.0	1.3	14.0
3DOe1(a)	2.1	48.3	1.3	44.3	1.3	23.3
	2.3	44.3	1.7	38.3	1.3	25.0
	1.3	41.3	1.4	41.3	1.4	26.4
	6.3	134.3	4.4	124.3	4.1	103.3

934 TABLE 5 (Continued)

	Alfalfa		Burr Clover		Fenugreek	
Strain	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen	Dry Weight	Total Nitrogen
	1.7	33.3	1.4	28.0	1.5	34.5
3DOe1(d)	1.6	34.6	1.3	40.1	1.7	37.7
	1.8	38.9	2.2	54.0	1.7	38.6
	5.1	104.8	4.9	134.1	4.9	110.8
	1.9	36.9	1.1	23.5	.3	7.3
3DOC3	1.5	34.1	1.3	36.6	.6	8.7
	1.8	37.0	1.6	37.7	.7	8.1
	5.3	108.3	4.0	105.1	1.8	35.6
	1.0	21.3	.3	12.0	.4	6.1
3DOC4	1.0	21.4	1.3	32.0	.5	8.0
	1.1	22.4	1.1	25.4	.3	4.5
	3.1	69.4	3.3	89.4	1.8	18.6
	.3	12.9	1.4	28.3	.4	5.9
3DOd2	1.3	32.0	1.3	32.0	.5	6.1
	1.4	25.4	1.3	25.9	.4	5.3
	3.4	68.3	4.3	103.3	1.3	16.6
	2.4	48.5	1.4	31.9	1.1	20.7
3DOf1	2.3	58.0	1.3	34.3	1.3	23.4
	2.9	55.0	1.5	36.8	1.5	27.7
	7.3	159.0	4.5	103.5	3.9	70.8
	1.3	32.0	1.0	24.9	1.7	32.4
3DOf2	1.3	44.0	.3	16.5	1.3	27.3
	1.8	35.3	.3	16.9	1.7	26.3
	5.3	117.3	1.7	58.0	5.9	75.5
	2.3	48.0	1.3	32.5	1.3	30.6
3DOf2(b)	1.9	50.1	1.9	47.5	1.6	30.3
	2.3	46.0	1.3	49.9	1.7	32.1
	6.3	144.1	5.0	129.5	5.1	88.9
	2.3	38.3	.3	8.9	.3	4.3
3DOe3	1.5	41.9	.5	11.4	.3	5.5
	2.1	42.0	.3	8.3	.3	4.3
	6.1	121.3	1.5	32.7	.9	14.1
	1.9	43.3	1.3	33.9	1.4	24.3
3DOZ1	1.9	46.1	1.3	41.9	1.3	27.3
	1.3	28.0	1.4	36.4	1.5	22.9
	5.5	121.4	5.3	120.4	4.3	74.7
	.7	13.5	.4	4.7	.4	6.4
Control	.8	12.7	.3	5.3	.3	5.5
	.5	15.2	.3	3.7	.3	5.0
	1.9	41.5	1.0	12.7	1.0	16.9
	.7	16.1	.3	5.9	.5	6.1
Control	.8	10.5	.3	3.0	.4	4.3
	.7	13.7	.4	4.3	.3	5.3
	2.0	40.0	.9	11.2	1.2	11.6

Each datum based on 10 plants.

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Delaware College

Agricultural

Experiment Station

A Preliminary Report on Muck Humus As a Fertilizer and Carrier of Beneficial Soil Bacteria

BY THOMAS F. MANNS AND JOSEPH M. GOHEEN

Newark, Delaware

DEFTS. EX. 16 IDEN.

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A Preliminary Report on Muck Humus As a Fertilizer and Carrier of Beneficial Soil Bacteria

A Preliminary Report on Muck Humus as a Fertilizer and Carrier of Beneficial Soil Bacteria

By Thos. F. Manns and Joseph M. Gourley*

Within recent years practical agriculturalists over the United States have come to recognize the ease and efficiency with which muck soils lend themselves to the production of truck crops. The utilization of mucks and peats for fertilizer fillers, materials for amending the physical condition of upland soils, and their use as a direct fertilizer has likewise served to bring the consideration of such material to the foreground.

It is recognized also that there may be as much difference between soils of organic origin as between the most diversified of the mineral soils. The commercialization of deposits of different formation has also served to emphasize this point thru the highly satisfactory results obtained in some cases and the injurious effects in others.

Micro-biological studies of American soils have been confined almost exclusively to those of mineral origin, but the increasing interest in organic soils and the important rôle that micro-organisms play in them make highly legitimate a study of their activities.

The object of these investigations was to determine (1) whether high grade muck or so-called "humus," such as that found in the trucking district in northern New Jersey, when properly inoculated, would carry beneficial soil bacteria, including the nodule organisms for the different agricultural and floral legumes; also whether reinforcement and composting of the muck humus would further increase or favor a beneficial flora. (2) Further, to determine the simplest method of adding such inoculation. (3) To determine whether one compost may carry the inoculant for all the agricultural and floral legumes at the one time and be sufficiently reinforced and balanced to make a high grade soil inoculator, renovator, stimulant and fertilizer. The work necessitated a study of the flora found in the virgin muck, and the changes brought about by successive years of cul-

* Holder of research fellowship given by the Alphano Humus Co., of New York City.

tivation, and further, what organisms should be added to make the material a general and beneficial soil inoculant.

The Status of Legume Inoculation

It has been known for a number of years that the organism causing nodules on certain legumes would also produce nodules on closely related species; for example, the organism producing nodules on white sweet clover (*Melilotus alba*) and yellow sweet clover (*Melilotus officinalis*) is the same as that which works on alfalfa. This same organism also works on Indian yellow sweet clover (*Melilotus indica*), trefoil or black medick (*Medicago lupulina*), yellow alfalfa (*Medicago falcata*), burr clover (*Medicago denticulata*), spotted medick (*Medicago arabica*) and *Trigonella foenum graecum*.

It is known that in the clover group the organism which inoculates red, mammoth, white and alsike clovers (*Trifolium pratense, T. medium, T. alba, T. hybridum*) also inoculates crimson, buffalo, rabbit-foot, hop, and hop trefoil clovers (*Trifolium incarnatum, T. reflexum, T. arvense, T. agrarium, T. procumbens.*)

Likewise the organism which covers the vetch and pea group, covers the plants of the genera Vicia, Pisum and Lathyrus, including many species.

A fourth group may be known as the bean group which covers many species.

At present, soy bean (*Soja spp.*) and cow pea (*Vigna spp.*) are apparently distinct, making six specific groups covering our important American agricultural and floral legumes.

Two other groups are those including the lupines and the lotus. M. Klummer and R. Kruger*, two European workers and H. Garman and M. Didlake** of Kentucky have recently published articles showing the groups into which certain of the different legumes fall.

Using this as a basis, we have gathered inoculation representing each of the above groups for the purposes of this work.

Variations in Composition and Flora of Muck and Peat

The investigations have necessitated the study of an inferior and very acid peat, for a comparison of the acidity, the chemical analysis, and the flora. Such a comparison was made with a peat being used

*Centbl. Bakt 2 Abt 40 (1914) No. 11-13-pp. 256-265.

**Bul. 184 (1914) Agr'l Expt. Sta. Kentucky, pp. 343-363.

in the vicinity of Sumner, New Jersey, which was quite coarse and poorly decomposed.

TABLE I—Comparison of Alphano, New Jersey, muck, with peat from Sumner, New Jersey

	Alphano, N. J. muck	Sumner, N. J. peat
(1)		
Lime Requirement (per 2,000,000 lb. acre, air dry basis.)	4200 to 5300 lbs. (CaO)	12,600 to 17,000 lbs. (CaO)
Nitrogen	2.67%	1.3%
(2)		
Humus or soluble Org. matter	26.2 to 29.4	4.3—6.8
Ammonifiers, per gram, dry basis.	50,000—260,000	33,333
(3)		
Nitrifiers, per gram dry basis.	10,000— 33,000	None.
(4)		
Azotobacter, per gram, dry basis.	20,000— 66,000	Trace.
(5)		
B. radicicola, per gram dry basis.	50,000—133,000	Trace.
Various moulds,	50,000—133,000	Trace.

(1) Lime requirement by the saturated calcium acetate method mentioned in a paper read before section C (Chem.) of the American Association for the Advancement of Science, Philadelphia, Pa., Dec. 31, 1914.

(2) Humus or soluble organic matter by the Grandeau method.

(3) By the author's medium No. 20 (see p. 37).

(4) By Ashby's medium modified with author's soil extract.

(5) By Ashby's medium—The Radicicola present were of the clover group.

Proposed Methods for Investigation

The methods proposed for carrying out, in the greenhouse, the composting, inoculation of the muck and testing for nodule production were as follows: Four gallon glazed earthen-ware jars were filled with 10,000 grams of muck containing optimum water conditions. After making the composts this amount was found to overfill somewhat the jars, accordingly the amount placed in each jar was 8,000 grams. In making the composts, the muck was used in about the condition it is piled; that is, containing about 65% moisture. All composts were computed on a ton basis. With the exceptions of the com-

posts numbers 9, 10, 11, 12, and 13 to which cyanamid was added and 23 and 24 to which guano was added, no nitrogen was used, excepting the small amount contained in the soil required for inoculation. Inoculation was carried out by using soil containing a well balanced flora including the nodule organisms of the different groups of valuable agricultural and floral legumes.

The inoculated soil was added in the proportion of 25 lbs. per ton of muck. A stimulant to increase certain groups of soil flora was added in the form of a crude carbohydrate, at the rate of 30 lbs. per ton, except in composts Nos. 2B, 3B, 4B, 5B, 6B, 7B and 8B, where 60 lbs. per ton were used.

In measuring inoculation or the efficiency of the compost for nodule production, four of the most important groups of legumes were grown in each compost,—viz., cow peas—soy beans—alfalfa and crimson clover, and a careful study made of the roots. Further, to study the value of the compost as a general soil inoculator, an inoculation in sterilized sand was carried out, using the compost in different amounts on a two million pound acre basis, viz., two lbs., 100 lbs. and 500 lbs. Five different legumes were employed, each representing an individual group, viz., alfalfa, red clover, winter vetch, cow peas and soy beans. To obtain data on the availability of nitrogen, a growth of rye and millet was used. Some chemical data were taken on nitrates which showed marked variations in the different composts. The fluctuations and variations in the flora were studied by plate cultures, using media that were adapted to bring out the more important physiological groups, such as the ammonifiers, the nitrifiers and the azotofiers, including *B. radicicola*. In making notes on the flora, only radical or marked changes were recorded.

Data for Building Composts

In order to build neutral composts from various alkaline, acid and neutral products such as the commercial phosphates, unleached hard wood ashes, cyanamid, various carbonates, lime, etc., when added to humus, the following experiments were carried out; various quantities of lime were added to the humus from Alphano, New Jersey to determine amounts required to neutralize the product. It was found that 4.5 to 7 pounds of calcium oxide will completely neutralize a ton on an air dry basis; that one pound of CaO will neutralize five pounds of 14% commercial acid phosphate. It was found that cyana-

mid is very caustic, equalling lime, and that one pound of cyanamid will neutralize five pounds of commercial 16% acid phosphate. One-tenth pound of CaO will neutralize one ton of sheep manure, and .11 pound of CaO will neutralize a ton of dried blood; one pound of unleached hard wood ashes (chestnut) will neutralize five pounds of commercial 16% acid phosphate. It requires about one pound of Thomas slag to neutralize one pound of commercial 16% acid phosphate.

From the above data, some 40 different composts have been made, running to extremes both on the basic and acid sides.

The Flora of Humus from Alphano, N. J.

The studies have been carried out on muck from a composite of the first four feet in digging. It is to be expected that on a muck that has been used for trucking purposes for two or three seasons prior to digging, many of the beneficial soil organisms will be present and active. On the trucking areas and the dikes at Alphano, clover plants showing an abundant inoculation with the nodule organism were observed. Under such conditions, it would be expected that some *B. radicicola* of the clover group would be present. This was shown to be true by the clover grown in the non-inoculated jars—A, B, and No. 29. (See Plate III). The non-inoculated muck showed no sign of the nodule organisms for cow peas, soy beans, alfalfa and vetch.

Plate cultures made with the various media used in these experiments showed that *B. radicicola* of the clover group was present, varying from 50,000 to 133,000 per gram of moist peat. Various azotobacters were present varying in number from 20,000 to 66,000. The nitrifying organisms were the lowest of all in number, varying from 10,000 to 33,000 per gram of moist peat. The ammonifiers were present from 50,000 to 260,000 per gram. Moulds were very plentiful, varying from 50,000 to 260,000.

A rich and well balanced soil flora would show all these organisms increased by ten to fifty fold, excepting the moulds which in neutral soils are not very plentiful. In the light sandy soils of lower Delaware, moulds to the number of 100,000 per gram of moist soil, have been taken. From sick soil in North Dakota *Fusarium lini*, the fungus causing flax wilt, has been taken to the extent of 45,000 colonies per gram of moist soil.

TABLE II—*Showing Nature of Compost, Amount of Nodule*

11/17/15: Started following composts		11/20/15: Planted this date: cow peas, soy beans, crimson clover and alfalfa. 1/11/16: All plants removed and nodules and growth observed.	
		Nodules	*Growth*
A			
Humus	2,000 lbs	Cow peas	none	good
		Soy beans	"	"
		Crim. clover	many	"
		Alfalfa	few (see Comp. No. 29)	"
B			
Humus	2,000 lbs.	Cow peas	none	good
Carbohydrate	30 "	Soy beans	"	"
		Crim. clover	few	"
		Alfalfa	none	"
C			
Humus	2,000 lbs.	Cow peas	none	good
Inoc. soil	25 "	Soy beans	"	"
		Crim. clover	many	"
		Alfalfa	some	"
D			
Humus	2,000 lbs.	Cow peas	none	good
Carbohydrate	30 "	Soy beans	some	"
Inoc. soil	25 "	Crim. clover	"	"
		Alfalfa	few	"
No. 1A			
Humus	2,000 lbs	Cow peas	none	slightly chlorotic
Acid Phos. (16%)	50 "	Soy beans	"	slightly chlorotic
$Ca(OH)_2$	20 "			
Organic ash	100 "	Crim. clover	few, scattered	slightly chlorotic
Carbohydrate	30 "			
Inoc. soil	25 "	Alfalfa	good, scattered	slightly chlorotic
No. 1B			
Humus	2,000 lbs.	Cow peas	none	chlorotic
Acid Phos. (16%)	50 "	Soy beans	"	"
Organic ash	200 "	Crim. clover	very few	"
Carbohydrate	30 "	Alfalfa	good	"
Inoc. soil	25 "			

Production, Growth of Legumes and Notes on the Bacterial Flora.

1/14/16: Replanted all composts with cow peas, soy beans, crimson clover and alfalfa. 3/30/16: Pulled all plants to study nodules and growth.

Study of flora on media Nos. 22, 23, 42, 43A, 43B, 44A, 44B, 45A,45B, and 46.

Nodules	*Growth*	*Notes on Bacterial flora*
well scattered	good	Used last six media above. *B. radicicola* very few,—many fungi—flora low in all media.
none	"	
general	"	
general	"	
well scattered	good	Flora fairly good—some Azotobacters; some *B. radicicola*—no molds.
slight	"	
quite general	"	
scattered	"	
well inoculated	good	Very poor flora; few *B. radicicola*, like A.
quite general	"	
abundant	"	
quite general	"	
some inoc.	good	Flora good—*B. radicicola* plentiful. However, nothing like as plentiful as in No. 8A, No. 8B, et al. Much the best check.
general	"	
very good	"	
very good	"	
good	good	Not an abundant flora—some Azotobacters and *B. radicicola*
none	"	
few	"	
good	"	
none	fair	Many Azotobacters; not many *B. radicicola*.
none	"	
few, but large	good	
few, but large	good	

TABLE II—(Continued).

11/17/15: Started following composts		11/20/15: Planted this date: cow peas, soy beans, crimson clover and alfalfa. 1/11/16. All plants removed and nodules and growth observed.		
			Nodules	*Growth*
No. 2A		Cow peas	some	good
Humus	2,000 lbs.			
Organic ash	100 "	Soy beans	"	"
Carbohydrate	30 "	Crim. clover	few	"
Inoc. soil	25 "	Alfalfa	good	"
No. 2B		Cow peas	none	poor
Humus	2,000 lbs.			
Organic ash	200 "	Soy beans	"	"
Carbohydrate	60 "	Crim. clover	"	good
Inoc. soil	25 "	Alfalfa	good	good
No. 3A		Cow peas	none	chlorotic
Humus	2,000 lbs.	Soy beans	none	"
Carb. of potash	50 "	Crim. clover	few	"
Acid Phos.	50 "	Alfalfa	fair	"
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 3B		Cow peas	(no growth—Carbonate of Potash too	
Humus	2,000 lbs.			
Carb. of Potash	100 "	Soy beans	strong)	"
Acid Phos.	50 "	Crim. clover		"
Carbohydrate	60 "	Alfalfa		"
Inoc. soil	25 "			
No. 4A		Cow peas	none	roots not good
Humus	2,000 lbs.			
Carb. of Potash	15 "	Soy beans	"	roots not good
Calcium Carbonate	10 "			
Acid Phos.	50 "	Crim. clover	abundant	good
Carbohydrate	30 "			
Inoc. soil	25 "	Alfalfa	"	good
No. 4B		Cow peas	none	none
Humus	2,000 lbs.			
Carb. of Potash	30 "	Soy beans	"	none
Calcium Carbonate	20 "			
Acid Phos.	50 "	Crim. clover	no inoc.	poor
Carbohydrate	60 "			
Inoc. soil	25 "	Alfalfa	good inoc.	fair

TABLE II—(Continued).

1/14/16: Replanted all composts with cow peas, soy beans, crimson clover and alfalfa. 3/30/16: Pulled all plants to study nodules and growth.		Study of flora on media Nos. 22, 23, 39, 43A, 43B, 44A, 44B, 45A, 45B, and 46.
Nodules	*Growth*	*Notes on Bacterial flora*
good	fair	Some few *Az. chroococcum* and many other Azotobacters. Many *B. radicicola.*
few	"	
few	good	
few	"	
none	fair	An abundant flora—many *B. radicicola* and *Azotobacters*
"	"	
"	poor	
good	"	
none	very poor	Many Azotobacters—few *B. radicicola*—a fairly good flora.
"	" "	
"	" "	
"	" "	
none	very poor	Some Azotobacters, but not as many as No. 3A.
"	almost no growth	
"	" " "	
"	" " "	
fairly good	fair	Many *B. radicicola, many* Azotobacters—fairly good flora.
" "	"	
very good	good	
splendid	"	
none	plants poor	An abundant flora. Many Azotobacters and many *B. radicicola.*
"	"	
some	" "	
none	" "	

TABLE II—(Continued).

11/17/15: Started following composts

No. 5A	
Humus	2,000 lbs
Potassium Carb.	30 "
Acid Phos.	50 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 5B	
Humus	2,000 lbs
Potassium Carb.	60 "
Acid Phos.	50 "
Carbohydrate	60 "
Inoc. soil	25 "
No. 6A	
Humus	2,000 lbs
Sodium Carb.	30 "
Acid Phos.	50 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 6B	
Humus	2,000 lbs
Sodium Carb.	60 "
Acid Phos.	50 "
Carbohydrate	60 "
Inoc. soil	25 "
No. 7A	
Humus	2,000 lbs
Thomas slag	200 "
Carbohydrate	30 "
Inoc. soil	25 "

11/20/15: Planted this date: cow peas, soy beans, crimson clover and alfalfa. 1/11/16: All plants removed and nodules and growth observed.

	Nodules	*Growth*
Cow peas	none	good
Soy beans	some	"
Crim. clover	abundant	"
Alfalfa	abundant	good
Cow peas	none	poor
Soy beans	"	poor
Crim. clover	quite abundant	quite healthy
Alfalfa	inoc. good	quite healthy
Cow peas	none	poor
Soy beans	"	"
Crim. clover	abundant	healthy
Alfalfa	"	"
Cow peas	(No growth—Sodium carbonate too strong.)	
Soy beans		
Crim. clover		
Alfalfa		
Cow peas	quite abundant	good
Soy beans	quite abundant	"
Crim. clover	quite abundant	"
Alfalfa	quite abundant	"

TABLE II—(Continued).

2/14/16: Replanted all composts with cow peas, soy beans, crimson clover and alfalfa. 3/30/16: Pulled all plants to study nodules and growth.		Study of flora on media Nos. 22, 23, 39, 43A, 43B, 44A, 44B, 45A, 45B, and 46.
Nodules	*Growth*	*Notes on Bacterial flora*
very slight	fair	Many *Azotobacter chroococcum*, also other Azotobacters; an abundant flora, exceptionally abundant in *B. radicicola*.
poor	"	
good inoc.	good	
splendid inoc.	"	
none	fair	An abundant flora—many *Azotobacter chroococcum*, and other Azotobacters; many *B. radicicola*. A good flora.
"	"	
slight	good	
good inoc.	"	
none	poor	Many Azotobacters, tho not as abundant as in 6B.
"	"	
very slight	"	
" "	"	
no growth	no growth	Abundant *Azotobacter chroococcum*. Many urea bacteria—probably *B. fluorescens*.
" "	" "	
" "	" "	
" "	" "	
splendid	good	Many Azotobacters—*B. radicicola* not abundant.
very good	"	
small and scattering	"	
" " "	"	

TABLE II—(Continued).

11/17/15: Started following composts

No. 7B	
Humus	2,000 lbs.
Thomas slag	100 "
Carbohydrate	60 "
Inoc. soil	25 "
No. 8A	
Humus	2,000 lbs.
Thomas slag	50 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 8B	
Humus	2,000 lbs.
Thomas slag	15 "
Carbohydrate	60 "
Inoc. soil	25 "
No. 9	
Humus	2,000 lbs.
Cyanamid	10 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 10	
Humus	2,000 lbs.
Cyanamid	25 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 11	
Humus	2,000 lbs.
Cyanamid	200 "
Acid Phos.	200 "
Carbohydrate	30 "
Inoc. soil	25 "

11/20/15: Planted this date: cow peas, soy beans, crimson clover and alfalfa. 1/11/16: All plants removed and nodules and growth observed.

	Nodules	*Growth*
Cow peas	none	fair
Soy beans	"	"
Crim. clover	quite abundant	"
Alfalfa	quite abundant	"
Cow peas	abundant	fine
Soy beans	"	"
Crim. clover	"	"
Alfalfa	"	"
Cow peas	many	fair
Soy beans	3 on 3 plants	"
Crim. clover	many	"
Alfalfa	"	"
Cow peas	none	healthy
Soy beans	"	"
Crim. clover	"	"
Alfalfa	few	"
Cow peas	none	none
Soy beans	none	none
Crim. clover	none	sickly
Alfalfa	"	"
Cow peas	none	none
Soy beans	none	none
Crim. clover	none	none
Alfalfa	none	none

TABLE II—(Continued).

1/14/16: Replanted all composts with cow peas, soy beans, crimson clover and alfalfa. 3/30/16: Pulled all plants to study nodules and growth.	Study of flora on media Nos. 22, 23, 39, 43A, 43B, 44A, 44B, 45A,45B, and 46
Nodules *Growth*	*Notes on Bacterial flora*
general inoc. fine	Many Azotobacters and *B. radicicola*—a good flora.
fair "	
good "	
scattered "	
good inoculation fine	A very abundant flora—many Azotobacters and many *B. radicicola*.
" " "	
scattered "	
" "	
good fine	A very abundant flora—many *B. radicicola* and Azotobacters—No. 43A is a good medium, and 43B also.
some "	
scattered "	
fair "	
none weak	An abundant fungus flora has overrun all six media.
" "	
scattered, large "	
none "	
none very weak	Same as No. 9.
" " "	
" " "	
" " "	
no growth no growth	Most abundant bacterial flora of all. Probably *B. fluorescens*.
" " " "	
" " " "	
" " " "	

TABLE II—(Continued).

1/11/16: Started following composts		1/14/16: Planted composts to cow peas, soy beans, alfalf and crimson clover. 3/30/16: Pulled all plants to study nodules and growth.		
			Nodules	*Growth*
No. 12		Cow pea	none	weak
Humus	2,000 lbs.	Soy beans	"	"
Cyanamid	10 "	Crim. clover	scattered	"
Acid Phos.	50 "	Alfalfa	"	good
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 13		Cow pea	none	weak
Humus	2,000 lbs.	Soy bean	"	"
Cyanamid	20 "	Crim. clover	"	none
Acid Phos.	100 "	Alfalfa	scattered	good
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 14		Cow pea	none	weak
Humus	2,000 lbs.	Soy bean	1 nodule	"
Organic ash	25 "	Crim. clover	scattered, large	"
Sodium Carbonate	15 "	Alfalfa	"	medium
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 15		Cow pea	scattered	fair
Humus	2,000 lbs.	Soy bean	slight inoc.	weak
Organic ash	25 "	Crim. clover	good inoc., large	good
Sod. Carbonate	15 "	Alfalfa	scattered, large	"
Acid Phos.	50 "			
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 16		Cow peas	none	none
Humus	2,000 lbs.	Soy beans	scattered	weak
Floats	400 "	Crim. clover	none	none
Sod. Carbonate	15 "	Alfalfa	"	weak
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 17		Cow peas	none	weak
Humus	2,000 lbs.	Soy beans	splendid	medium
Floats	400 "	Crim. clover	none	none
Potas. Carbonate	15 "	Alfalfa	good inoc.	good
Carbohydrate	30 "			
Inoc. soil	25 "			

TABLE II—(Continued).

Study of Flora on media Nos. 22, 23, 25, 39, 43A, 43B, 44A, 44B, 45A, 45B, and 46.

Notes on Bacterial flora

Plate C—(C—1/100,000 gram) after two days incubation—45 colonies. Plate C—Medium 46—after 4 days, 800 to 1000 colonies—very few Azotobacters, some *B. radicicola*. Mostly urea bacteria.

Plate C—after 2 days incubation—20 colonies. Plate C—medium 46—after four days, 700 to 900 colonies probably urea bacteria.

Plate C—after two days incubation—25 colonies. Plate C—medium 46—after 4 days incubation—250 colonies, many Azotobacters and *B. radicicola*.

Plate C—after 2 days incubation—30 colonies. Plate C—medium 46—after 4 days incubation—about 275 colonies, mostly Azotobacters and *B. radicicola*.

Plate C—after 2 days incubation—30 colonies. Plate C—medium 46—after 4 days incubation—200 colonies—some Azotobacters and *B. radicicola*.

Plate C—after two days incubation—75 colonies. Plate C—medium 46—after 4 days incubation, 150 colonies—many Azotobacters and *B. radicicola*.

TABLE II—(Continued).

1/11/16: Started following composts

No. 18	
Humus	2,000 lbs.
Floats	100 "
Sod. Carbonate	15 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 19	
Humus	2,000 lbs.
Organic ash	100 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 20	
Humus	2,000 lbs.
Organic ash	50 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 21	
Humus	2,000 lbs
Organic Ash	25 "
Carbohydrate	30 "
Inoc. soil	25 "
No. 22	
Humus	2,000 lbs.
Organic ash	50 "
Calcium Carbonate	50 "
Carbohydrate	30 "
Inoc. soil	25 "

1/14/16: Planted composts to cow peas, soy beans, alfalfa and crimson clover. 3/30/16: Pulled all plants to study nodules and growth.

	Nodules	*Growth*
Cow peas	scattered, small	fair
Soy beans	scattered, small	medium
Crim. clover	none	none
Alfalfa	good	weak
Cow peas	good	good
Soy beans	abundant, large	"
Crim. clover	plentiful, big	"
Alfalfa	scattered, big	"
Cow peas	abundant inoc.	very good
Soy beans	very strong	very good
Crim. clover	abundant, large	very good
Alfalfa	very strong	very good
Cow peas	some inoc.	good
Soy beans	medium inoc.	"
Crim. clover	medium inoc.	"
Alfalfa	good inoc.	"
Cow peas	none	none
Soy beans	abundant inoc.	strong
Crim. clover	abundant inoc.	very strong
Alfalfa	good	very strong

TABLE II—(Continued).

Study of Flora on media Nos. 22, 23, 25, 39, 43A, 43B, 44A, 44B, 45A, 45B, and 46.

Notes on Bacterial flora

Plate C—after 2 days incubation—45 colonies. Plate C—medium 46—after 4 days incubation, 200 colonies—Azotobacter and *B. radicicola.*

Plate C—after 2 days incubation has 50 colonies. Plate C—medium 46—after 4 days incubation; 100 colonies, large proportion probably Azotobacters—some *B. radicicola.*

Plate C—after 2 days incubation has 20 colonies. Plate C—medium 46—after 4 days incubation—150 colonies—some *B. radicicola* and Azotobacters.

Plate C—after two days incubation—30 colonies. Plate C—medium 46—after four days incubation—200 colonies—many small Azotobacters and *B. radicicola* colonies.

Plate C—after two days incubation—50 colonies. Plate C—medium 46—after four days incubation, 250 colonies, some Azotobacters, and many *B. radicicola.*

TABLE II—(Continued).

1/11/16: Started following composts		1/14/16: Planted composts to cow peas, soy beans, alfalfa and crimson clover. 3/30/16: Pulled all plants to study nodules and growth.	
		Nodules	*Growth*
No. 23			
Humus	2,000 lbs.	Cow peas	few, small	medium
Guano	10 "	Soy beans	few, large	strong
Calcium Carbonate	50 "	Crim. clover	abundant, small	good
Carbohydrate	30 "	Alfalfa	few, small	"
Inoc. soil	25 "			
No. 24				
Humus	2,000 lbs.	Cow peas	small nod.	small
Guano	10 "	Soy beans	very few	"
Organic ash	50 "	Crim. clover	abundant, small	"
Carbohydrate	30 "	Alfalfa	none	none
Inoc. soil	25 "			
No. 25				
Humus	2,000 lbs.	Cow peas	abundant, very large	very healthy
Pot. Carbonate	10 "	Soy beans	good, large	very healthy
Acid Phos.	50 "	Crim. clover	medium, small	very healthy
Calcium carbonate	100 "	Alfalfa	abundant, very large	very healthy
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 26				
Humus	2,000 lbs.	Cow peas	few	medium
Potassium Carb.	10 "	Soy beans	abundant, large	healthy
Thomas slag	25 "	Crim. clover	plentiful small	"
Calcium carb.	100 "	Alfalfa	scattered, medium size	small
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 27				
Humus	2,000 lbs.	Cow peas	abundant, medium	large, healthy
Calcium carb.	100 "	Soy beans	abundant, large	very healthy
Carbohydrate	30 "	Crim clover	none	none
Inoc. soil	25 "	Alfalfa	scattered, medium size	small

TABLE II—(Continued).

Study of Flora on media Nos. 22, 23, 25, 39, 43A, 43B, 44A, 44B, 45A, 45B, and 46.

Notes on Bacterial flora

Plate C—after two days incubation—450 probably nitrifying bacteria. Plate C—medium 46—after four days incubation, 100 colonies—many nitrifying bacteria.

Plate C—after two days incubation—900 colonies—probably nitrifying bacteria. Plate C—medium 46—after four days incubation, 500 colonies—probably nitrifying bacteria.

Plate C—after two days incubation—100 colonies. Plate C—medium No. 46—after 4 days incubation—200 colonies—some Azotobacters—few *B. radicicola.*

Plate C—after two days incubation—30 colonies. Plate C—medium No. 46—after four days incubation—200 colonies—many Azotobacters—many *B. radicicola.*

Plate C—after two days incubation—50 colonies. Plate C, medium 46—after four days incubation—150 colonies, some Azotobacters and *B. radicicola.*

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TABLE II—(Continued)

1/11/16: Started following composts		1/14/16: Planted composts to cow peas, soy beans, alfalfa and crimson clover. 3/30/16: Pulled all plants to study nodules and growth.		
			Nodules	*Growth*
No. 28		Cow peas	abundant, large	very healthy
Humus	2,000 lbs.	Soy beans	abundant large	plants healthy
Organic ash	25 "	Crim. clover	exceedingly abundant	splendid
Thomas slag	25 "	Alfalfa	scattered but large	small
Cal. Carb.	100 "			
Carbohydrate	30 "			
Inoc. soil	25 "			
No. 29		Cow peas	none	healthy
Humus	2,000 lbs.	Soy beans	"	"
		Crim. clover	good inoc.	"
		Alfalfa	none	"
No. 30		Cow peas	abundant inoc.	healthy
Humus	2,000 lbs.	Soy beans	abundant inoc.	"
Carbohydrate	30 "	Crim. clover	abundant inoc.	"
Inoc. soil	25 "	Alfalfa	good inoc.	"

TABLE II—(Continued).

Study of Flora on media Nos. 22, 23, 25, 39, 43A, 43B, 44A, 44B, 45A, 45B, and 46.

Notes on Bacterial flora

Plate C—after two days incubation—60 colonies. Plate C—medium 46—after four days incubation—300 colonies, many Azotobacters, probably *A. chroococcum*, many *B. radicicola*.

Plate C—after two days incubation—5 colonies. Plate C—medium 46—after four days incubation—50 colonies, some Azotobacters, few *B. radicicola*.

Plate C—after two days incubation—50 colonies. Plate C, medium 46, after four days incubation—150 colonies, mostly Azotobacters and *B radicicola*.

The Flora in the Different Composts

Remarkable changes were brought about in the muck by the application of various ingredients. The different applications of wood ashes, sodium carbonate and potassium carbonate greatly increased the *Azotobacters*, especially the species *A. chroococcum*. (See the dark colonies in plate X).

The influence of basic materials in every compost was to reduce molds, but in some cases they increased *Actinomyces*. This observation is in harmony with studies that have been made on agricultural soils, especially those having large amounts of alkaline salts, such as potassium and sodium carbonates. One of the soils from Colorado, high in basic compounds, showed ten million *Actinomyces albus* per gram. This number is far in excess for this organism from soils which are neutral or acid.

The influence of the carbohydrate was to bring up immediately an abundant *Azotobacter* flora, the species varying with the amount and nature of the basic materials applied. As stated above, the basic materials containing alkaline compounds greatly favored *Azotobacter chroococcum*. The inhibiting action on moulds of a properly balanced compost is seen by comparing cultures A and B, Plate VIII, in which plate A is from a compost that has been properly balanced by alkaline bases. The influence of lime and other bases was very marked in the great increase of *B. radicicola*. In some cases the increase was a hundred to a thousand fold or more. The increase in *Azotobacters* was frequently a thousand fold. The rapid disappearance of moulds was remarkable. They evidently died out, as they failed to come up in the culture media when plating. The most remarkable influence of the bases was upon the nitrifiers, here these minute organisms which carry out such an important work, showed a wonderful increase. The importance of these biological processes and the part played by lime and the alkaline compounds must not be underestimated.

In the composts containing cyanamid and guano, especially the former, marked increases were noticeable in the urea bacteria. The most abundant flora was found in the composts containing cyanamid. Here, a plating from a dilution of 1/100,000 gram of moist compost was not sufficient to permit of proper development. Over 900 colonies of urea bacteria, probably *Bact. fluorescens* per 1/100,000 gram of moist compost were counted or rather estimated in a plate.

Symbiosis or Mutualism Probably an Active Factor with Azotobacters and B. radicicola

Thruout this work there is abundant evidence that there exists a strong mutualism between different species of *Azotobacter* and *B. radicicola*. It was observed that in all the media favorable for growth of *Azotobacter*, the same was very suitable for *B. radicicola;* in fact Ashby's medium proposed for Azotobacters, (Jour. of Agr. Science II.—1907-8—p. 54), was equally a splendid medium for *B. radicicola*. It was observed that wherever a colony of *B. radicicola* started on a plate, that when *A. chroococcum* or other azotobacters became a part of the colony the rapidity of growth for both was very marked. When *Azotobacters* are carried in tube cultures they live and make splendid growth when they are associated with *B. radicicola*. The *B. radicicola* shows an equal benefit from the mutualism. It is believed that in the older root tubercles of legumes, *B. radicicola* is rapidly followed up by certain *Azotobacters*. This appears certain from the many *Azotobacters* which have found their way into old but apparently sound nodules, as indicated by surface sterilizing and plating. Many years of study of *B. radicicola* on pure culture and its rapid deterioration and failure, and on the other hand great success when carried in soil has led to the conclusion that symbiosis or rather mutualism is a great factor in preserving and benefitting a soil flora.

It has been inferred by certain producers of commercial inoculants that the organism of each particular legume requires a culture medium slightly different from that of another, and particularly adapted for the organism in question; further that "the germs for one variety of legumes will not work on any other variety of legume." In the light of recent investigations, the present work shows that the organisms for the different groups of the important agricultural legumes may be successfully carried in one and the same compost.

The Influence of Various Compounds on Inoculation

The influence of the composition of the compost is apparently not so active on the soil flora as it is on the host. This was especially to be noted on the growth of the different legumes. In many instances when the roots of the legumes failed to penetrate deeply into the compost because of excessive acidity or alkalinity, the few roots which were to be found showed an abundant inoculation. On the other hand, in certain composts where the nitrogen available was too

great, altho the legumes showed splendid growth and a bountiful root system, little or no inoculation was to be found. It required strong acidity and alkalinity in the compost before a marked falling off in the *B. radicicola* content could be detected in cultures. On the other hand, a slight excess of sodium and potassium carbonates, either from that applied in organic ash or when applied in the form of chemically pure goods, would show detrimental effect on soy beans and cow peas. However, red clover and most especially alfalfa were quite tolerant to alkali. Alfalfa was in every case the last to die out from excessive alkalinity. The influence of an excess of these carbonates was to be seen on the growth of soy bean and cow pea in composts numbers 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 14, 15, 16, 18 and 19. Because of the detrimental effect of these alkalies on soy beans and cow peas in some of these composts no inoculation apparently took place. (See Table II, composts 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5B 6A and 6B) Some of these same composts gave splendid inoculation on clover and alfalfa (See Table II, composts 4A-5A). The size and apparent health of the nodule was very noticeable on clover and alfalfa grown in some of these sodium and potassium carbonate treated pots. The influence of fertilizers carrying nitrogen, such as cyanamid and guano, was to inhibit or weaken inoculation. This was plainly seen in composts 12, 13, 23, and 24, the two former carrying cyanamid nitrogen, and the two latter, nitrogen from a high grade guano.

Statements have been made that the nodule organisms do not live well in an acid soil. In pot C and Pot No. 30, we have added inoculation but no bases; the results were a very good inoculation on all the legumes, soy beans, cow peas, clover and alfalfa and this in a humus that shows a lime requirement of nearly 5,000 pounds of CaO per 2,000,000 pound acre. (See plate IV showing photograph of inoculation in pot 30). The growths of the legumes on pots C and 30 were also fairly good, showing that these plants were able to tolerate an acidity in the form of humus, that was nearly three times that of the average acid soil.

The best inoculations were found in those composts which were well balanced in their make-up, such as composts Nos. 8, 25 and 28. Here there is not only an abundant and well distributed inoculation, but also plant growth which shows health and vigor. See plates V and VI for the inoculation in No. 25 and No. 28 and plate II for growth of legumes on these composts.

Inoculation in Sterilized Sand By Use of Composts

This work was carried out in 7-inch plant pots, sterilizing the sand in the autoclave four hours, at a pressure of fifteen pounds. The composts were used at the rates of 3 pounds, 100 pounds and 500 pounds respectively to a 2,000,000 pound sand acre. (This would be equivalent to about a six inch acre). Five different legumes were employed, representing as many different nodule organism groups, viz., alfalfa, clover, vetch, cow peas and soy beans. This work was done in duplicate. The sand was treated with ground limestone and washings from wood ashes to supply the necessary minerals. The work was duplicated, using two different composts for inoculation. The appearance of the legumes was somewhat better where 100 pounds and 500 pounds respectively of the composts were used, but upon examination, it was found that good inoculation had taken place in all the pots, and among all the legumes. (See Plate VII.) This work readily indicates that the organisms for each and all of the important groups of agricultural legumes may be carried in one compost. An observation which was of particular interest in this work was the immense amount of root growth in the sand cultures as compared with the top growth on the legumes. The same relationship must take place in the growth of legumes on weak sandy soils. The following shows dry weights in grams, of tops and roots from the series inoculated with 100 pounds of compost per acre.

Dry Weights of Tops and Roots of Legumes Grown in Sand Cultures.

	Wt. of Tops	Wt. of Roots
Alfalfa	5.1 grams	12 grams
Clover	4. "	12 "
Vetch	4.5 "	11 "
Cow Peas	7.2 "	10 "
Soy Beans	6. "	9 "

The splendid inoculation produced in these sand cultures would indicate that such humus as was used in these experiments, could readily be inoculated and composted so as to carry all the important organisms for the inoculation of the different groups of important agricultural legumes.

The Influence of the Basic Compounds on the Availability of Humus Nitrogen

The importance of basic compounds in making available the nitrogen of humus has long been known. Certain of the elements, such as calcium, magnesium, iron, manganese and aluminum form insoluble humates in the soils, while others such as sodium and potassium, especially the carbonates of these and the compound ammonia, have the property of making humates soluble. Humus or probably more properly speaking, humic acid has stronger affinity for sodium, potassium, iron, aluminum, and phosphorus than it has for calcium and magnesium. This is very evident when the Grandeau method for humus is applied, in which the calcium and magnesium are almost entirely taken out by the preliminary treatment with weak hydrochloric acid, while the other basic materials are not separated from the humus. This fact is plainly seen when the ash content of humus as reported by Snyder[1] (Hilgard, "Soils," p. 134, 1906), and given herewith for reference is observed.

Ash of Humus from Minnesota Prairie Soils

Insoluble matter	61.97
Potash (K_2O)	7.50
Soda (Na_2O)	8.13
Lime (CaO)	0.09
Magnesia (MgO)	0.36
Peroxide of Iron (Fe_2O_3)	3.12
Alumina (Al_2O_3)	3.48
Phosphoric Acid (P_2O_5)	12.37
Sulfuric Acid (SO_3)	.98
Carbonic Acid (CO_2)	1.64

The cultural tests with rye and millet were used only as indicators to show whether small or large amounts of the humus nitrogen were being made available for the plants. The following table shows the crop response from rye both green and dry, which was harvested from the different composts after a growing period of eight weeks. (See Plates XI, XII and XIII).

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TABLE III

Showing Green and Dry Weights of Rye Grown Eight Weeks

Compost No.	Green Weight in Grams	Dry Weight in Grams
A check	63	11
B	88	16.5
C	56	11
D	117	25
1A	252	45
1B	312	45
2A	187	34.5
2B	257	38.5
3A	167	34
3B	78	10.5
4A	95	21.0
4B	220	40
5A	141	31
5B	169	32
6A	160	34
6B	130	23
7A	125	26.5
7B	125	24
8A	Pot removed before data were taken.	
8B	173	33.5
9	342	66
10	313	60.5
11	0	0
12	303	47
13	57	18
14	115	19
15	107	25.5
16	127	23
17	89	21
18	146	29
19	210	40
20	149	32
21	146	30
22	152	31
23	383	54
24	281	49

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TABLE III—(Continued).

Compost No.	Green Weight in Grams	Dry Weight in Grams
25	Pot removed before data were taken.	
26	139	28
27	73	14.5
28	100	22.5
29 check	50	11.5
30 check	75	16.5

From Table III, it is very apparent that the addition of certain basis materials has strong influence on the nitrogen assimilation. Check composts A and No. 29 show the response from humus not reinforced or inoculated with soil organisms; these gave a response to rye of 11 and 11.5 grams of dry material (See Plates XI and XIII.)

Check C shows that inoculation alone did not help any, giving a dry weight of only 11 grams; however, when a stimulant or bacterial food such as a crude carbohydrate is added, the pot, without inoculation, is increased by 5.5 grams or 50%, as seen in Check B. When such crude carbohydrate and inoculation are added, the increase is 14 grams or about 122%. Composts numbers 1A, 1B, 2A and 2B show the importance of organic ash, both with and without addition of acid phosphate. The two former have additions of acid phosphate, and show nearly 50% increase above the two latter, which have no additional phosphorus. The difference between Check D, and composts 2A and 2B respectively, shows the influence of organic ash, which is an increase of 9.5 grams and 13.5 grams, or when placed on a per cent basis, is 34% and 54%; the latter shows that when it comes to rye response that 200 pounds of organic ash per ton of humus is superior to 100 pounds. However, 50 pounds of acid phosphate, when added to the 200 pounds of organic ash per ton of compost, show a further increase, viz., the difference between 45 grams and 38.5 grams, or 6.5 grams, which is an increase of nearly 17%.

The detrimental effects of an excess of sodium or potassium carbonates are to be observed in various composts from 3A to 6B nd 14 to 18 respectively, in which certain pots made very poor growth, especially 3B, in which 100 pounds of potassium carbonate and 50 pounds of commercial 16% acid phosphate were used. Here, the dry weight was only 10.5 grams, which was 14.5 grams less than check D. Detrimental effects with 15 pounds or more sodium and potassium carbonates were observed unless the compost was properly balanced

with acid phosphate. Attempts to supplement applications of ashes with sodium or potassium carbonate resulted in decreased yields of rye, as seen by comparing compost No. 19, containing ashes which gave a dry weight of 40 grams, with composts Nos. 14 to 18 respectively, in which ashes were reinforced with sodium or potassium carbonates; here, the weights were 19, 25.5, 23, 21 and 29 grams respectively.

Calcium carbonate does not equal the response of wood ashes; this is seen by comparing compost No. 19, containing 100 pounds of ashes per ton of humus and producing a dry weight of rye of 40 grams, with compost No. 27 containing an equal amount of calcium carbonate per ton of humus, which gave a rye response of only 14.5 grams.

It is very difficult to make a comparison in humus pot cultures of the value of humus nitrogen with that of cyanamid and guano nitrogen; from the fact that the humus must be properly balanced before proper crop response can be expected. Such comparisons were attempted with cyanamid in pots Nos. 9, 10, 11, 12 and 13, in which pots No. 9 and No. 12 were supposedly well balanced composts. However, 10 pounds of cyanamid in a ton of humus, with no acid phosphate added, gave a higher rye response than did 10 pounds of cyanamid and fifty pounds of acid phosphate. It may be that where the acid phosphate was added, an inhibiting effect from this combination took place, although such was not the case when acid phosphate was used with ashes. The dry weights were respectively 66 grams and 47 grams. When cyanamid at the rate of 25 pounds per ton of humus was added, the crop response on rye was 60.5 grams dry weight as compared with 47 grams when 10 pounds of cyanamid and 50 pounds of acid phosphate were added. Ten pounds of cyanamid alone gave a higher rye response (66 grams dry weight) than did ten pounds of guano when the humus and the latter were balanced with 50 pounds of calcium carbonate or 50 pounds of ashes. (Compare pot 9 with pots 23 and 24).

The highest responses given to rye by the best humus composts to which no nitrogen was added, were found in composts 1A, 1B and No. 19, in which the dry weights were respectively 45, 45 and 40 grams. This is an increase over the untreated or uncomposted humus, using check A (11 grams dry weight) of 34, 34, and 29 grams respectively; in the two former the increases are over 300 per cent. Comparing this with the responses from the properly composted guano, which gave an increase over check A of 38 and 43 grams respectively, the response

from the humus nitrogen is a close competitor. The chief object, however, of adding cyanamid and guano nitrogen was not to obtain a comparison of crop response from these different sources of nitrogen, but to learn whether greater crop response could be brought about by such addition, and further whether cyanamid and guano were suitable sources for such reinforcement. Rye, in some cases, as noted above, gave slight additional increase from reinforcement with cyanamid and guano, while millet on the other hand gave greater response from twelve different composts, not reinforced with nitrogen, than from those pots receiving additional nitrogen from cyanamid and guano. The response from the cyanamid nitrogen in pot 9, viz., 66 grams dry weight, compares favorably with that from guano nitrogen in pot 23, which gave 54 grams dry weight. Here equal weights are being used; that is, 10 pounds of each in a ton, and the former is listed to contain nearly twice the amount of nitrogen as the latter. The guano was represented to be "genuine Peruvian Guano" having the following analysis:

Nitrogen	10.69%
Equivalent to Ammonia	13.00%
Available Phosphoric Acid	10.00%
Total Phosphoric Acid	11.00%
Actual Potash	3.00%

Crop Response from Millet

A study of the crop response from millet shows even more favorable results from the composts. None of the pots reinforced with cyanamid nitrogen or guano equalled the composts Nos. 1A, 1B, 7B and 22. In fact there were 12 different composts which gave higher crop production than did those reinforced with cyanamid and guano.

With the exception of compost No. 22, compost 1B was again the most productive, being closely followed by No. 7B and No. 1A. The dry weight with millet from these leading pots was over five times that from check A, while with rye it was only four times that of the check. No attempt will be made to interpret the response from the different composts. It is sufficient to indicate the compounds which appear to be very active in bringing about such response. Carbohydrates, apparently have great influence as there is a difference between raw humus in check A and that of humus and the crude carbohydrate in check B of 38 grams which is a response of nearly 176%. The addition of ashes to check D, as seen in pot 2A, gives 94 grams dry weight

or an increase from 65 to 94 grams, that is 29 grams, which is equal to 44.6% increase.

If compost 1B is compared with check A, it is evident that the addition of acid phosphate, ashes, the crude carbohydrate and inoculation changes the response from 22 grams, dry weight, to 116 grams or an increase of 472.7%.

Inoculation as shown in crop response in rye and millet has been the weakest factor at work, the increase from check A to check C amounting to three grams, that is the difference between 22 and 25 grams; this is an increase of only 13.6%. The increase produced by inoculation in the presence of a crude carbohydrate as seen in the increase from check B to check D is only 8.3%. Ashes and ground limestone more than doubled the crop as seen by comparing check 30 with pot 22 in which the former produced 55 grams and the latter 121 grams. The detrimental influence of an excess of potassium carbonate is seen in compost 3B, in which 100 pounds were added per ton of humus. In compost 6B, 60 pounds of sodium carbonate show a decidedly injurious effect. Table IV shows the green and dry weights of millet produced in eight weeks of growth on the different composts.

TABLE IV

Showing Green and Dry Weights of Millet Grown Eight Weeks

Compost No.	Green Weight in Grams	Dry Weight in Grams
A Check	105	22
B "	282	60
C "	125	25
D "	297.5	65
1A	377	113
1B	387.5	116
2A	352.5	94
2B	415	97
3A	310	75
3B	97.5	20
4A	192.5	34
4B	382	96.5
5A	387.5	106
5B	410	109
6A	253	63
6B	57.5	11
7A	248	52

TABLE IV—(Continued).

Compost No.	Green Weight in Grams	Dry Weight in Grams
7B	397	114
8A	Pot Removed	Pot Removed
8B	310	65
9	175.5	42
10	139	35
11	0	0
12	177	36
13	262.5	60
14	340	90
15	382.5	108
16	270	54
17	357.5	69
18	240	58
19	328	92
20	355	86
21	302.5	63
22	352	121
23	100	19
24	382	76
25	Pot removed	Pot Removed
26	340	75
27	165	29
28	355.5	85
29	114	22
30	257.5	55

Crop Response in Relation to Nitrification and Water Soluble Humus

It was thought that probably some light would be thrown upon the crop response by a study of nitrification. Accordingly, after harvesting the rye, the composts were carefully worked over, that is thoroughly mixed and moistened down; they were then kept at optimum conditions for two weeks when samples were removed for a study of nitrification. Twenty gram samples of the air dried composts were moistened with 150 cc of distilled water. After shaking for fifteen minutes, 100 cc of the water extract was filtered off and evaporated for nitrate determination. In some instances the color of the water extract was a very dark brown, showing that the treatment with the alkali carbonates was making the humus soluble. The following table

shows the color of the water extract, the reaction to litmus paper, and the amount of nitrate present in 20 grams of the air dry compost.

TABLE V

Showing Color of Water Extract, Reaction to Litmus Paper, and Amount of Nitrates found in each Compost

Number of Compost	Color of Water Extract	Reaction to Litmus	Milligrams of Nitrate Nitrogen in 20 grams of compost
A	light straw	acid	.0100
B	light straw	acid	.0200
C	light straw	acid	.0500
D	light straw	acid	.0150
1A	almost clear	alkaline	.0400
1B	darker than A	alkaline	.0300
2A	quite yellow	alkaline	0350
2B	strong cider yellow	alkaline	.0950
3A	yellowish brown	alkaline	.0350
3B	very dark brown	alkaline	2.5000
4A	lighter than A	almost neutral	.0100
4B	darker than A	alkaline	.0200
5A	darker than A	alkaline	.0150
5B	quite yellow	alkaline	.0400
6A	stronger than 5B	alkaline	.0250
6B	very dark brown	alkaline	.6000
7A	same as A	alkaline	.0200
7B	same as A	alkaline	.0250
8A	compost removed before	data were taken	
8B	same as A	alkaline	.0250
9	clearer than A	alkaline	3.6875
10	very clear	alkaline	10.6500
11	darker than A	alkaline	.0800
12	clearer than A	acid	5.6250
13	clearer than A	acid	8.5250
14	Red cherry brown	alkaline	.0650
15	same as A	alkaline	.0100
16	very dark brown	alkaline	.0350
17	cider brown	alkaline	.0200
18	dark brown	alkaline	.0250
19	quite yellow	alkaline	.0150
20	same as A	alkaline	.0100
21	lighter than A	alkaline	.0200
22	same as A	alkaline	.0150
23	lighter than A	alkaline	.0050

TABLE V—(Continued).

Number of Compost	Color of Water Extract	Reaction to Litmus	Milligrams of Nitrate Nitrogen in 20 grams of compost
24	little darker than A	alkaline	.1050
25	compost removed before	data were taken	
26	little darker than A	alkaline	.0100
27	same as A	alkaline	.0250
28	same as A	alkaline	.0100
29	same as A	acid	.0300
30	little darker than A	acid	.0250

From a study of Table V it is readily noticeable that composts showing almost no nitrate nitrogen gave splendid crop response with both rye and millet. It is difficult to know just how to interpret this. It is quite probable that other forms of nitrogen, than nitrate, cause ready crop response; and it may be possible that this form of nitrogen is not even the ammoniacal form.

The small amount of nitrate nitrogen in compost No. 11 would indicate that 200 lbs. of cyanamid per ton of humus is sufficient to stop entirely nitrification. On the other hand 25 lbs. of cyanamid per ton of humus shows strong nitrification. The composts containing guano showed less nitrate nitrogen than the check pots. It is hard to interpret this fact unless a denitrifying flora is at work.

Some Modifications of Media Used in This Work

For general plating of *B. radicicola* and *Azotobacters*, Ashby's medium has been used. (Jour. Agr. Sci. Vol. II, p. 54 1907-'08). It is prepared as follows:

Mannit	10.0 grams
Mono-potassium phosphate	0.2 "
Magnesium sulphate	0.2 "
Sodium chloride	0.2 "
Calcium sulphate	0.1 "
Calcium carbonate	5.0 "
Agar	12.0 "
Pure water	1000.0 cc

To favor a better growth of *Azotobacter, Nitrosococcus* and *Nitrobacter*, the following soil extract has been substituted in place of the water above. Take 500 grams of rich garden soil and add 2,000 cc of

water to which one gram each of ammonia tartrate and sodium citrate have been added. After vigorously shaking, the soil is allowed to settle; upon standing 24 hours the clear supernatant liquid is siphoned off. 1,000 cc of this soil extract is substituted for the water in Ashby's medium. (This medium is the senior author's medium No. 46).

To favor the nitrifiers (*Nitrosococcus* and *Nitrobacter*) one gram of ammonium sulphate per liter of medium was added to Ashby's medium with the above soil extract.

The following medium known as the author's medium No. 39 is of especial value in bringing out nitrifiers so they may be seen as brown colonies on the plates. In plating soils on this medium, large plates of 110 mm or larger in diameter and only 10 cc of medium are used, so that a thin layer of medium is formed. One-ten thousandth or one-one hundred thousandth gram of soil is used for each plate.

Mono-potassium phosphate	(KH_2PO_4)	4.0 Grams
Ferric sulphate		0.2 "
Wood ashes (hard)		12.0 "
Mannit		10.0 "
Agar		15.0 "
Soil Extract (Manns', as noted above)		1000.0 cc

After tubing above (10 cc to a tube) add to each tube one gram of the following insoluble mixture, the ingredients of which have been passed thru a sieve of 120 mesh to the inch.

Calcium limestone (ground rock)	100 grams
Kaolin	20 "
Gypsum	10 "
Dolomite	5 "
Rock phosphate (floats)	5 "

After liquifying the medium and just prior to plating, add to each tube 1 cc of a sterile ammonium sulphate solution made as follows,—one gram of ammonium sulphate in 100 cc of water. The object of adding the sterile ammonium sulphate just prior to plating is to prevent loss of ammonia which would take place if the ammonium sulphate was added with the other ingredients which are basic.

When adding the soil dilution in the process of plating, thoroly shake just before pouring so that the above insoluble minerals will be

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evenly distributed thruout the plate. It will form a gray mass or back ground on which the brown nitrifiers are very visible. For class work a qualitative test may be made around each nitrifying colony showing the plentiful formation of nitrates by a heavy blue coloration with the use of diphenylamine. This medium has been successfully used for several years by the senior author in class work.

Summary

1. Muck humus of the quality used in these experiments becomes, when properly balanced and reinforced, a very favorable medium for carrying a beneficial soil flora.

2. The flora of virgin muck humus was found low in many of the beneficial bacteria. Different legume nodule organisms were absent, though the clover group was present to a limited amount. The alfalfa, vetch-pea, bean, cow pea and soy bean groups were absent (see plate III).

3. Muck humus when properly reinforced, inoculated and composted will carry the several groups of the different legume organisms in one and the same compost, producing a strong inoculation on the different legumes.

4. Inoculation of the compost may be satisfactorily brought about by using soil which has been proved to give strong inoculation to each of the groups of legumes. *Azotobacter chroococcum* may be readily introduced by using western soil; soils from Colorado and North Dakota were used in these experiments. Other species of *Azotobacter* are common in eastern soils. Nitrifiers are common in all good soils.

5. Muck humus properly reinforced, inoculated and composted gave strong inoculation to five groups of legumes, when the latter were grown in sterilized sand cultures, using the compost at the rate of two pounds per a 2,000,000 pound acre. A somewhat stronger inoculation and growth were produced by using the compost at the rate of 100 and 500 pounds respectively to a two million pound acre in sand cultures (see plate VII).

6. Crude carbohydrates and basic compounds such as organic ash and Thomas slag proved very efficient in bringing up an active legume flora, and likewise a strong nitrogen fixing and nitrifying flora.

7. Organic ash proved the most active mineral application for use in the compost as indicated by crop response.

8. By properly reinforcing the composts with certain basic compounds, all the nitrogen required for crop response was made available as indicated by the growth of millet; the crop response from additional nitrogen from cyanamid and guano did not equal that from certain of the composts.

9. This work shows that the nodule organism may live well in a muck humus having a lime requirement of 5,000 pounds of CaO to a two million pound acre; also that in this same acidity four different legumes, viz. alfalfa, red clover, soy bean and cow pea, made good growth and took good inoculation without addition of any bases (see plate IV).

10. Muck humus as herewith reinforced and composted gave splendid crop response to rye and millet without the addition of nitrogen, indicating it to be a product of good fertilizing value.

Descriptions of Illustrations

Plate I—Showing four different legumes, viz., alfalfa, clover, cow pea, and soy beans, growing in the different composts to test same for inoculation.

Plate II—Same as plate I, showing four different legumes, viz., alfalfa, clover, cow pea, and soy beans growing in the different composts to test same for inoculation.

Plate III—Showing the amount of inoculation carried in the natural humus as dug from the trucking fields. The legumes were one month old when removed for photographing; (1) soy bean, (2) alfalfa, (3) crimson clover, (4) cow pea. The clover group is the only form of *B. radicicola* present.

Plate IV—Showing amount of inoculation taking place in one month's growth on soy bean, alfalfa, clover and cowpea, in natural humus to which inoculation but no minerals or bases have been added. A stimulant in the way of a crude molasses has been added. The inoculation is nearly equal to that shown in plates V and VI, to which bases have been added. Compare plate IV with plate III.

Plate V—Showing the amount of inoculation in compost No 25, in which bases have been employed. The soy beans, alfalfa, clover and

cow peas have taken a strong inoculation in one month. Compare with plate III.

Plate VI—Showing the amount of inoculation in compost No. 28 in which bases have been employed—all the legumes have taken a strong inoculation in one month.

Plate VII—Showing growth of five legumes, in sand culture, viz., alfalfa, crimson clover, vetch, cow peas and soy beans, representing as many different groups of *B. radicicola*. The amount of compost used was 2 lbs., 100 lbs. and 500 lbs. respectively. All showed strong inoculation indicating that in one compost all the groups may be successfully carried.

Plate VIII—Showing type of flora in natural humus (A) before composting and after composting (B). The molds and Actinomyces disappear and *Azotobacters*, *B. radicicola*, and nitrifiers become abundant. Culture A is from 1/10,000 gram of muck, and culture B is from 1/100,000 gram of muck.

Plate IX—Showing the bacterial flora from one-one hundred thousandths of a gram of soil, grown on Ashby's medium, comparing composts No. 20, 21, 22, 23 and 24, with the natural humus No. 29. A large increase in *Azotobacters* and *B. radicicola* has taken place.

Plate X—Showing the bacterial flora from one one-hundred thousandths of a gram of soil, grown on the author's medium, No. 46, adapted for *Azotobacters*. Here *Az. chroococcum* is shown plentiful in composts No. 1, 1-B, 6 and 6-B.

Plate XI—Showing growths of rye on the different composts. The influence of the bases is readily seen in composts 1-A and 1-B.

Plate XII—Continuation of work shown in plate XI—rye growing in different composts.

Plate XIII—Continuation of work shown in plates XI and XII —Rye growing in different composts.

Plate XIV—Showing color of water extract from different composts. Some were very dark brown. See Table V.

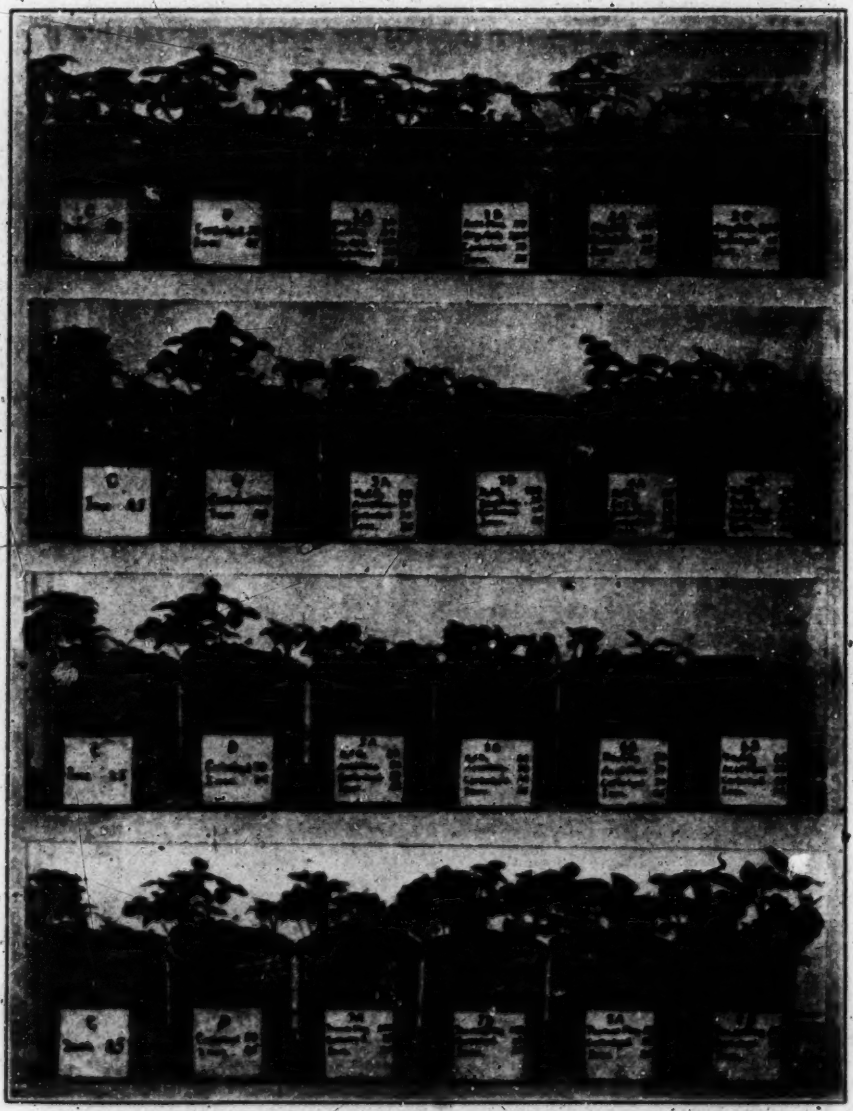


PLATE I.

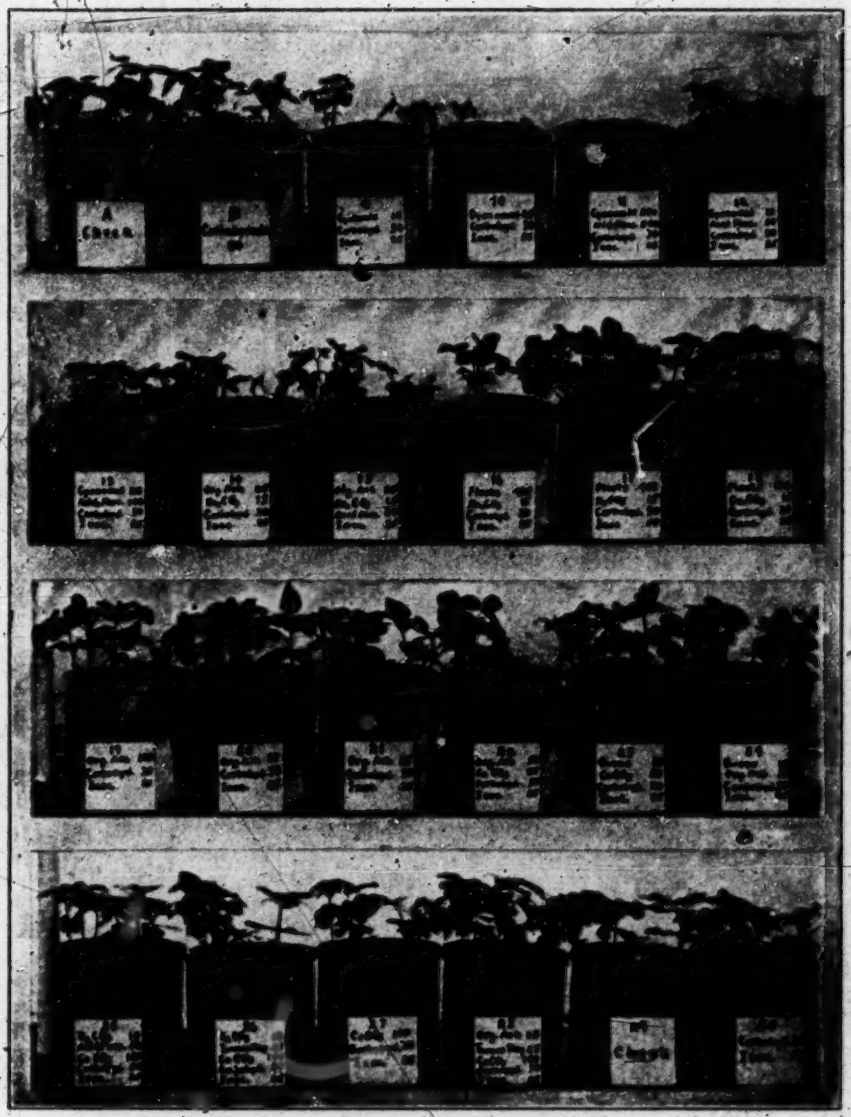


PLATE II

PLATE III

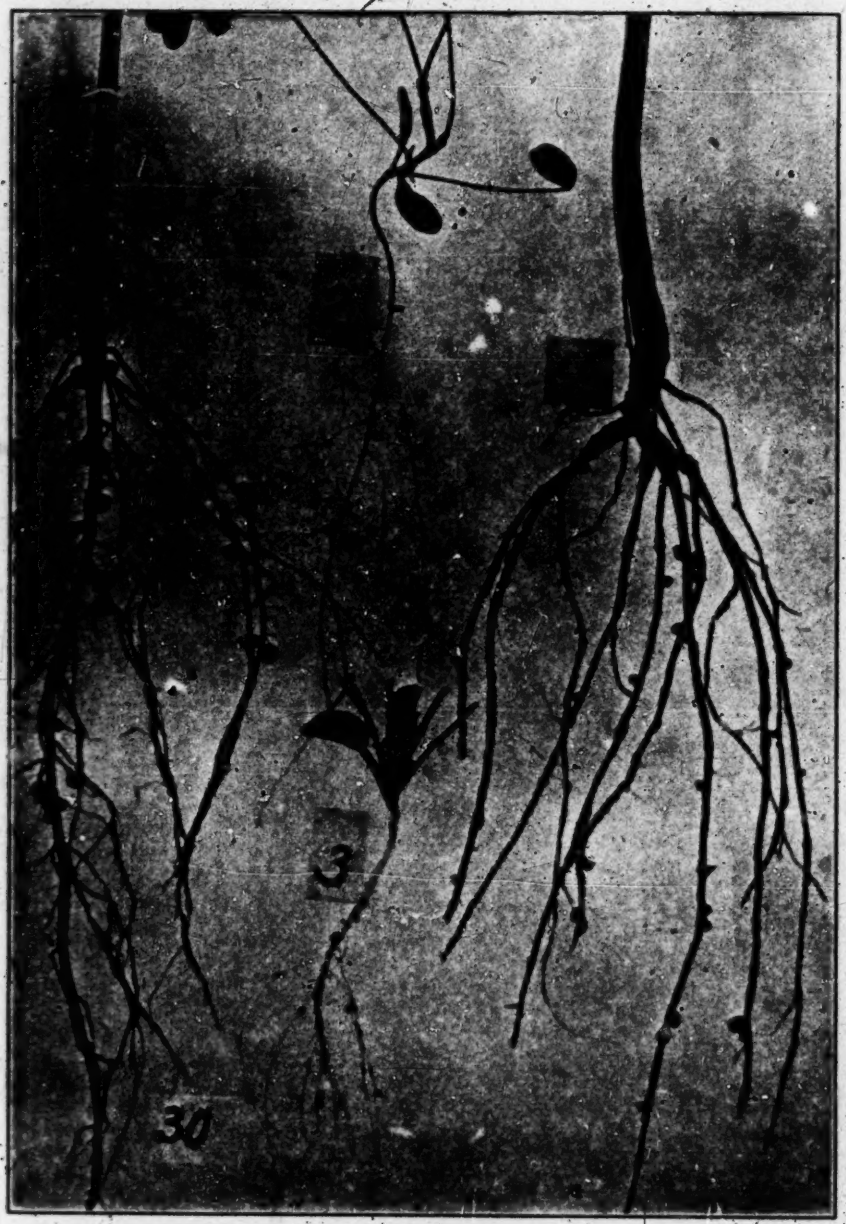


PLATE IV

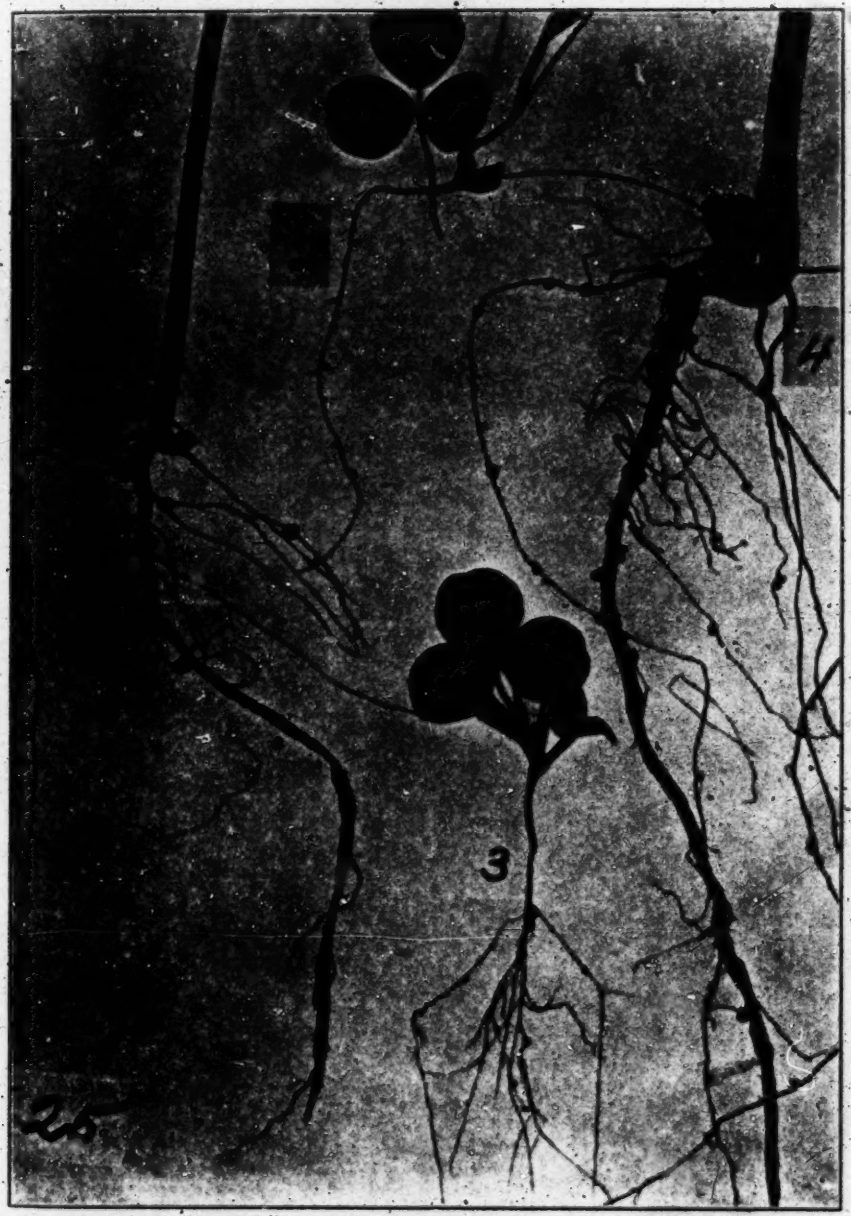


PLATE V

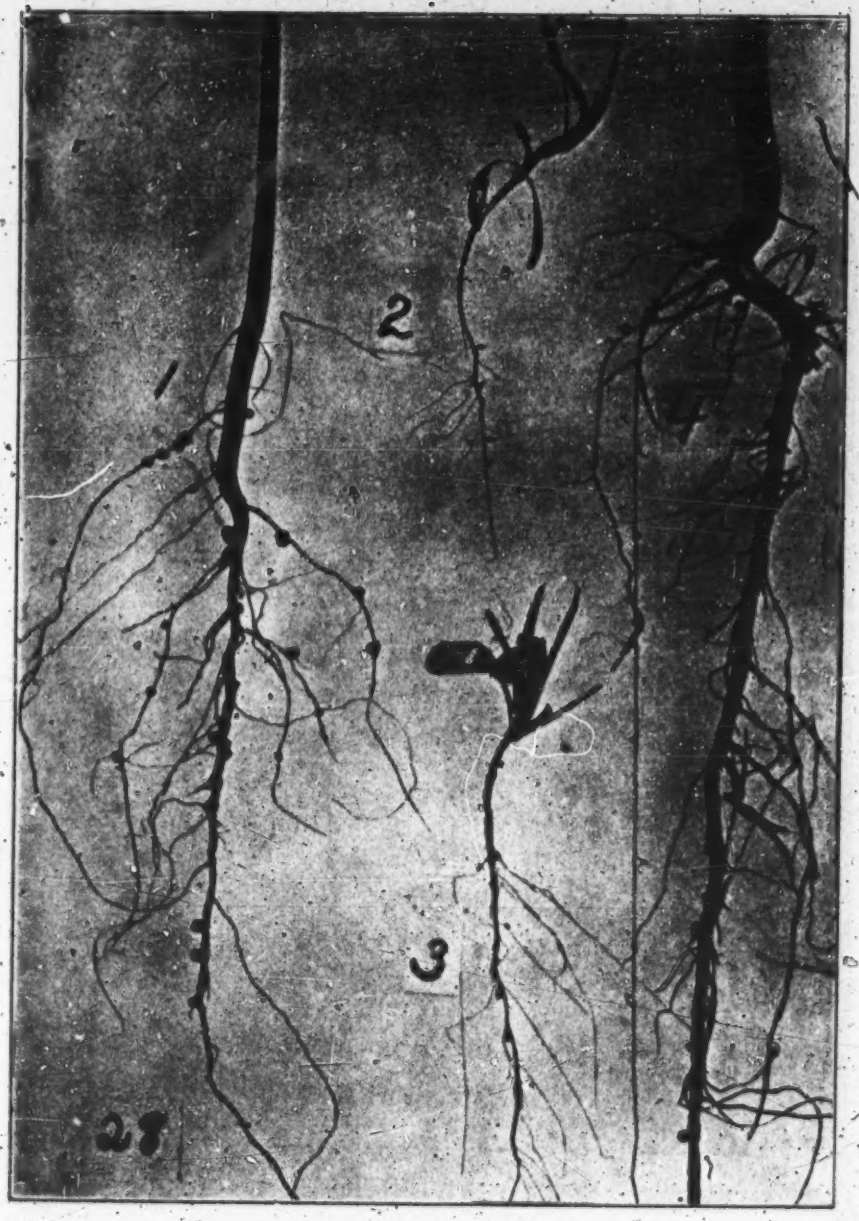
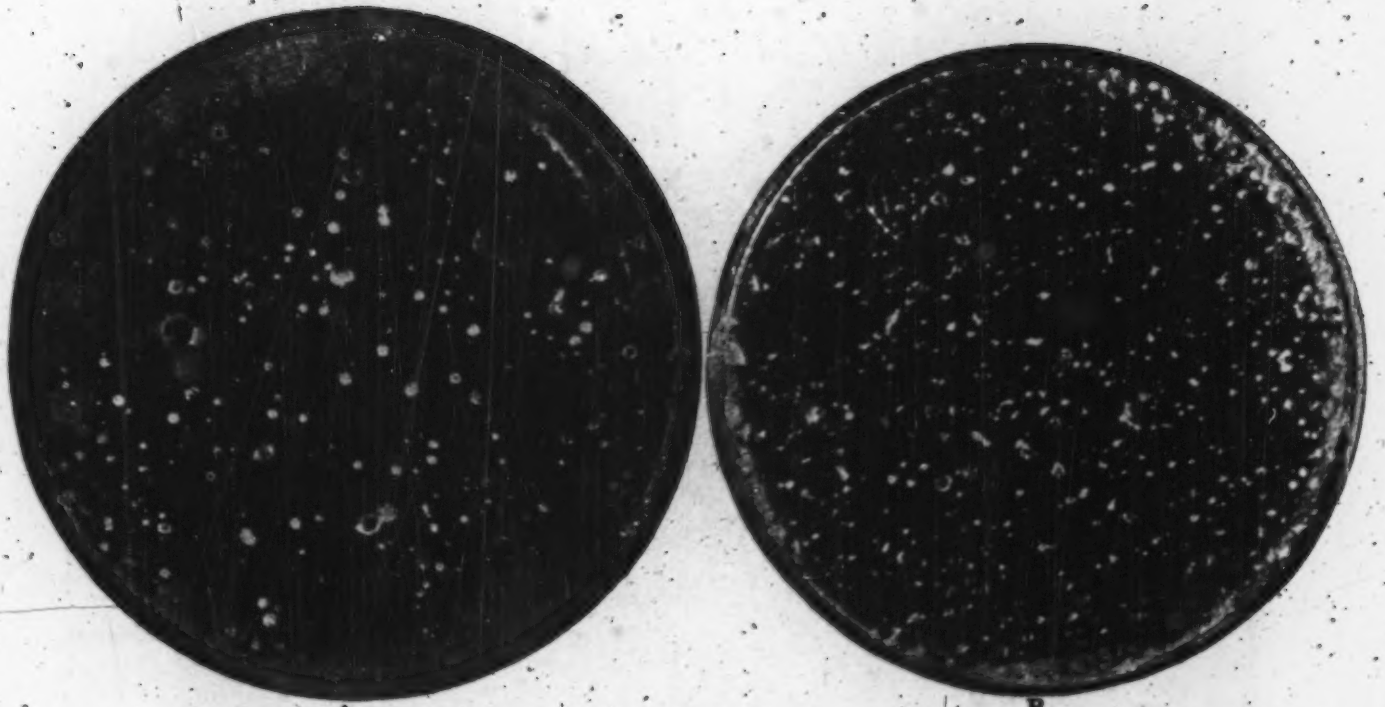


PLATE VI

PLATE VII



A B

PLATE VIII

PLATE IX

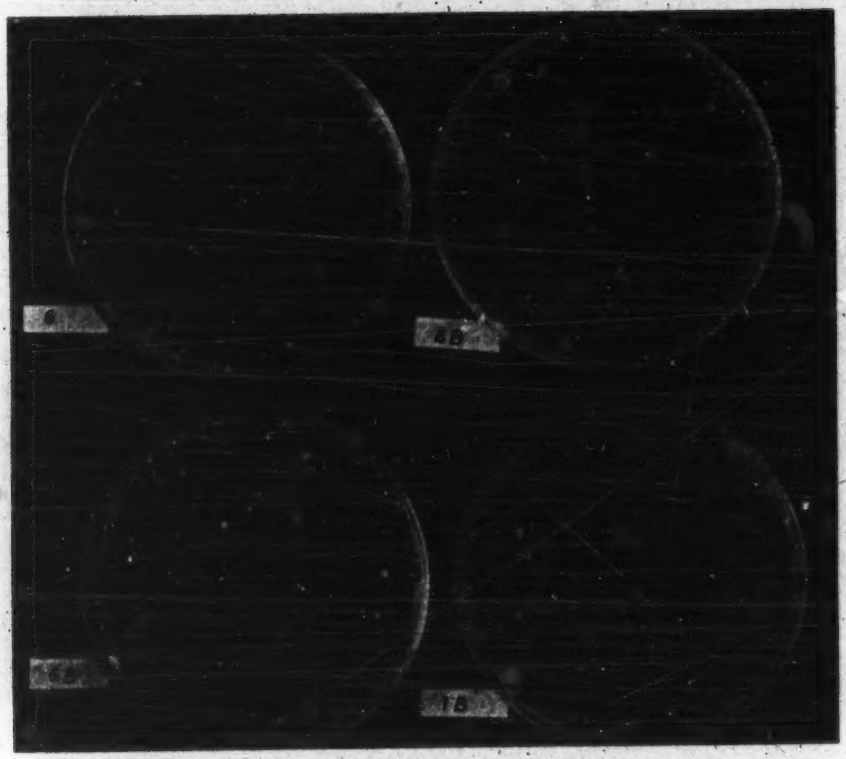


PLATE X

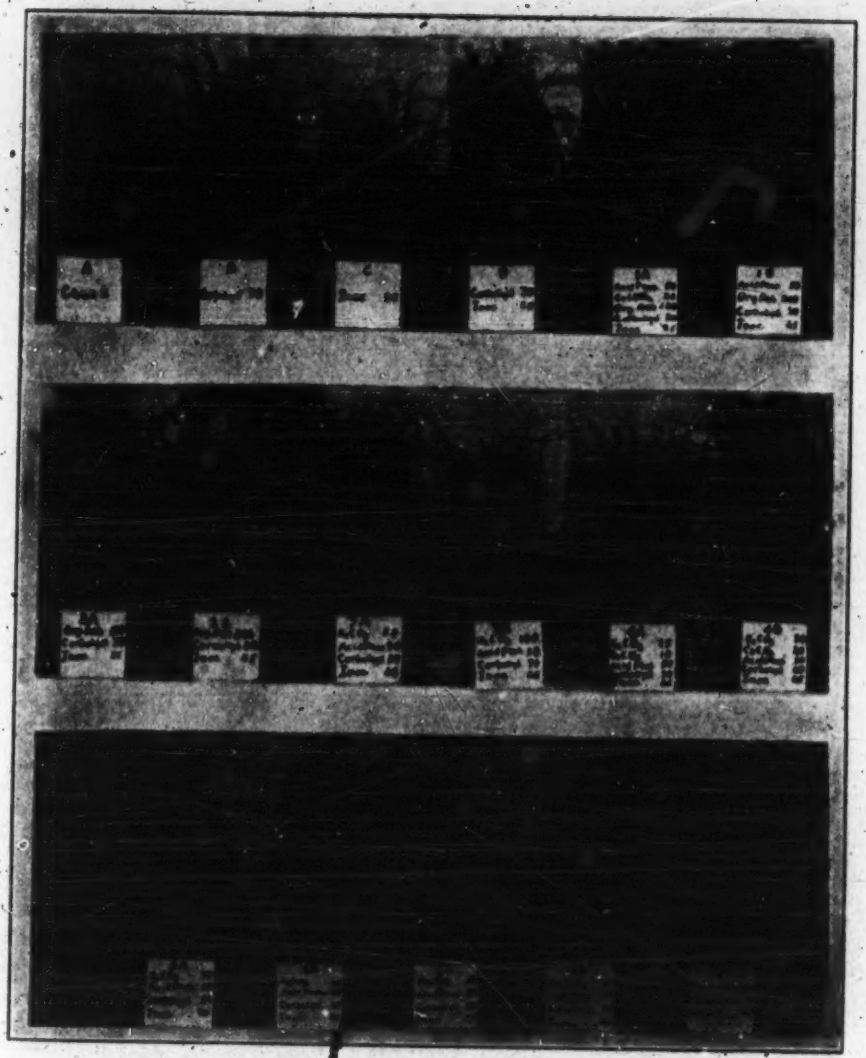


PLATE XI

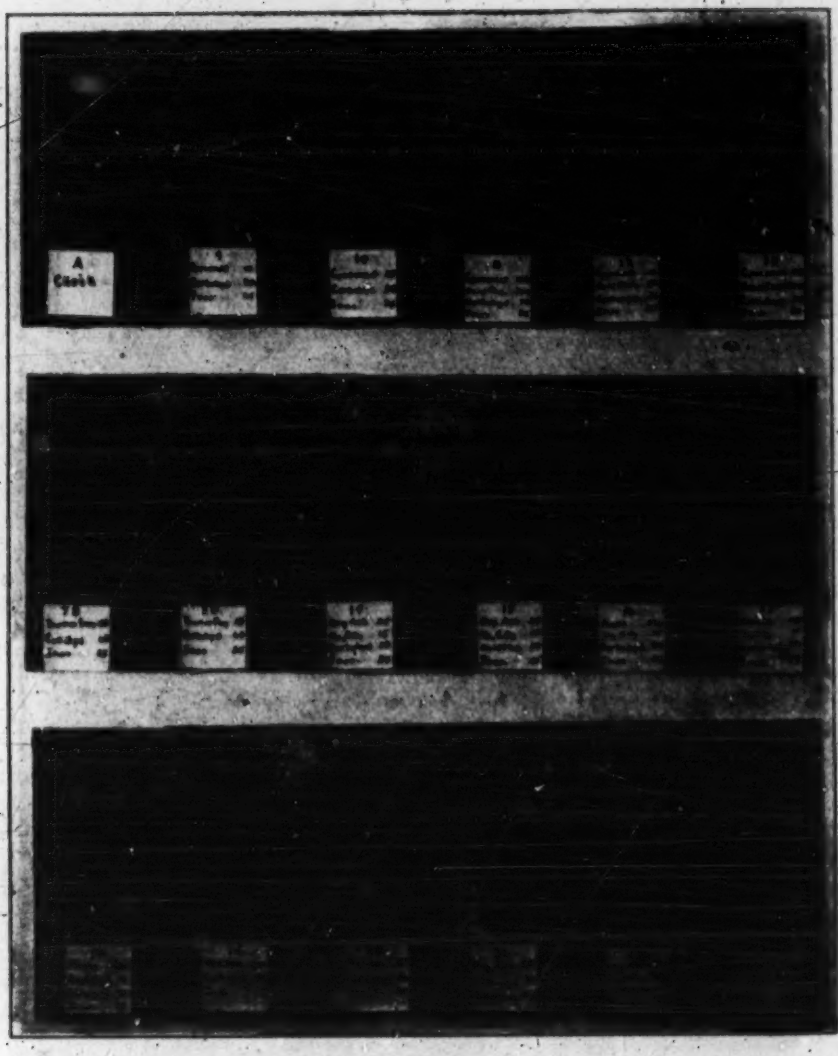


PLATE XII

PLATE XIII

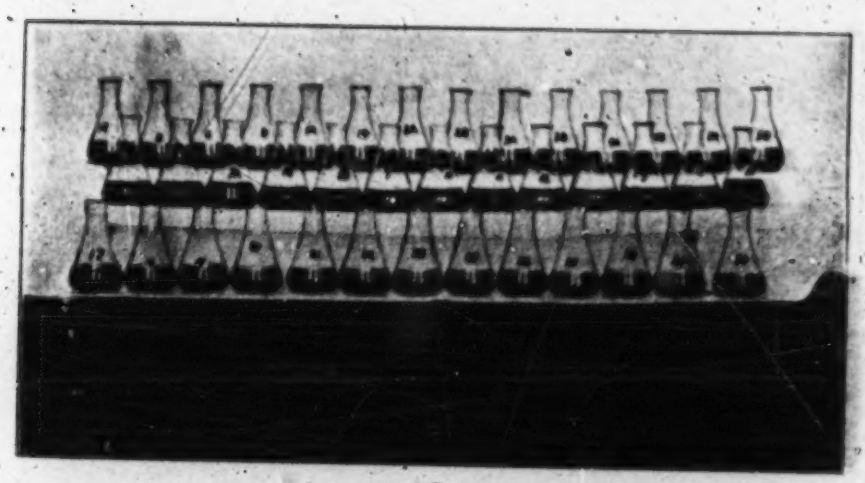


PLATE XIV

The Alphano Book

DEFTS. EX. 17 IDEN.

The Alphano Book

It has mainly to do with your soil and the best way to secure its highest fertility. With which are included some friendly hints on lawns, their care and fare; along with suggestions for your vegetables, flowers, shrubs and trees.

10 years on the market
Half a million tons sold.

Alphano Humus Co

Established 1905

Whitehall Building, 17 Battery Place
New York City

Subject Short Cut Guide

Page 3.

Soil Needs—A Short Talk on Them.

Page 4.

Natural Humus—An Explanation and a Caution.

Page 5.

Alphano—Its Strong Points.

Page 6.

For Lawns, Tennis Courts and Golf Links. What Alphano Will Do for Them.

Page 7.

Directions for Making New Lawns—Remaking Old Ones.

Pages 8-9.

Four Interesting Examples of Alphano Results with Flowers and Grass.

Page 10.

How to Use Alphano in both Your Flower and Vegetable Gardens.

Page 11.

Something About Peas, Beans, Celery and Some Interesting Points About Setting Out and Boosting Plants.

Page 12.

Two Letters Telling Phenomenal Results Secured from Alphano-Grown Potatoes and Peas, to Which Are Added Some Convincing Photographic Proofs.

Page 13.

Success with Clay and Sandy Soils, Along with Some Help Hints About Growing Hollyhocks.

Page 14.

Your Shrubs and Trees—What Alphano Will Do for Them.

Page 15.

Tree Planting and Feeding—Four Helpful Hints.

Page 16.

Rhododendrons—Why Alphano Makes a Happy Home for Them.

Page 17.

Your Potted Plants and Window-Boxes. A Sure Guide to Their Success.

Page 18.

For Reseiling Purposes—The Part Alphano Should Play.

Page 19.

The Greenhouse and the Way Alphano Is Revolutionizing Growing Methods.

Page 20.

Soil Inoculation. An interesting Explanation, to Which is Added Some Convincing Facts About Alphano Inoculant.

Page 21.

A Cheap, Sure Method of Multiplying the Value of Your Compost Heap, in Addition to Which There are Some Interesting Facts About Alfalfa and Timothy Growing.

Page 22.

Expert Advice on Soils and Grass Seeds, Besides a Suggestion for Securing a Soil and Humus Tester.

Page 23.

Prices, which are Accompanied by the Usual Terms—Method of Shipment and Akin Facts Which You Always Want to Know.

Page 24.

An Invitation to Visit Our Plant. Kindly Consider This Especially Directed to Yourself and Friends.

Dr. Milton Whitney's Convincing Statement

DR. MILTON WHITNEY, Chief of the U. S. Soil Bureau, in Bulletin No. 55, page 51, in speaking about chemical fertilizers and those of organic origin, says:

> "It would seem that for the greater number of soils the most efficient fertilizers of the future will be of organic rather than mineral origin, provided that when such a substance is found it can be obtained in sufficient quantity; and at a sufficiently low cost, and be at the same time less bulky to ship than stable manure."

Alphano Humus is such an organic fertilizer—and more.

Caution

For your protection, insist on ALPHANO when ordering Humus. Others offered under the name "Humus," bearing various trade designations, are apt to be mere swamp muck. These may not be worth $2.00 a ton, and of little or no plant food value; perhaps even harmful to plant life.

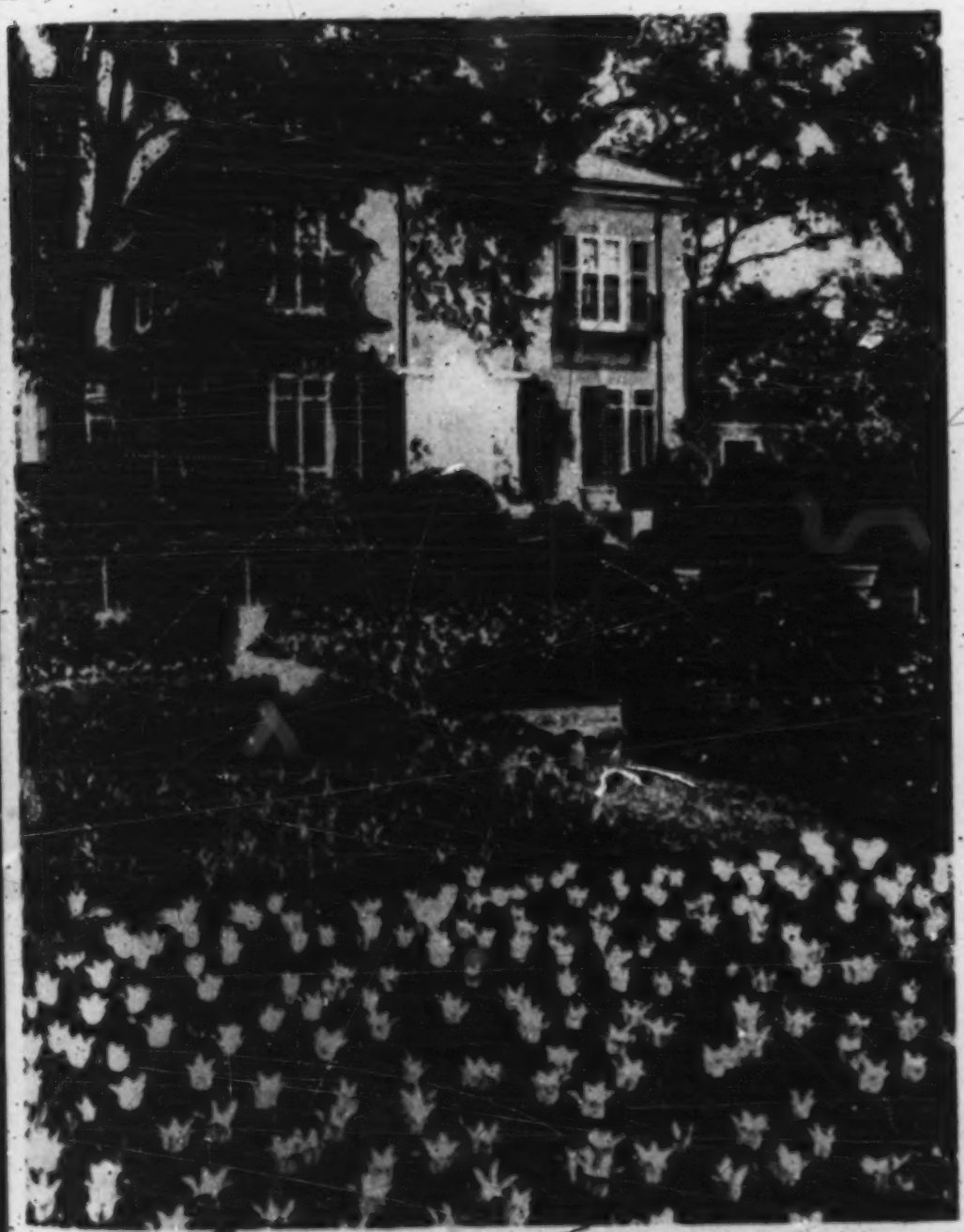
Guarantee

Take no chances. Ten years experience of thousands of satisfied Alphano users is your guarantee.

We stand back of every word we say, and every promise we make.

Alphano must and positively will give results.

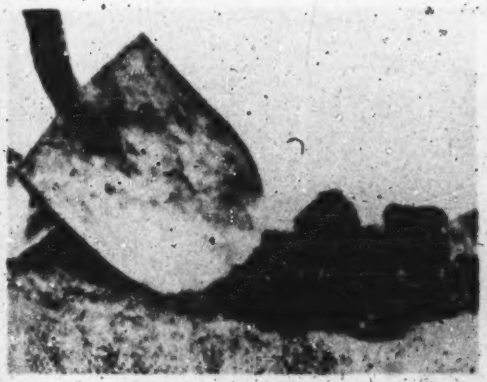
Enjoy the good results it is bound to bring to your lawn, shrubs, garden and greenhouse.



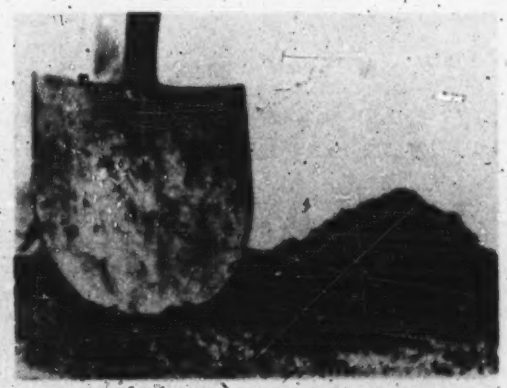
What is there which comes closer to our hearts in the early Spring than a garden like this, glorious with its bloom-coloring of tulips?

Their flowering period is short, but right up to the first of August growth is going on down in their bulbs. Upon the food and strength then stored up mainly depends their Spring bloom abundance. **Enrich them well with Alphano, as soon as the flowers are spent.**

This famed Long Island garden has luxuriated in Alphano for a number of years.



This lumpy, packy soil needs humus. Such soil is difficult to till, stays lumpy and lacks productiveness. It is made up of too much clay, and too little decayed vegetable matter, or humus.



The same soil, a week after being mixed with Alphano, which is rich in humus. Note how friable it is. Gardening with free working soil like this doubles both the pleasure and productiveness.

A Short Talk On Soil Needs

SOILS and people are a deal alike. No matter who we are, or what our work is, there must be in our food certain proportions of certain life-giving elements, else our blood becomes impoverished.

When a soil fails to produce it is because of either lack of food or food unfitted for the plants' needs.

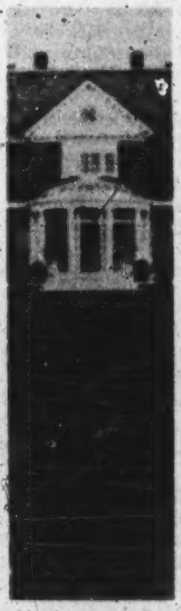
The first thing to find out about your soil is whether it is acid or alkaline. If acid, it needs sweetening with lime. We will gladly tell you a simple, quick way to find this out. See page 22.

It is a well understood fact that the great difference between sterile subsoil and fertile top soil is the difference in the humus they contain. It's apparent, then, that humus is the very backbone of soil productiveness.

Decayed vegetable matter is crude humus.

Barnyard manures, in addition to their plant foods, contain a percentage of humus, but their food values are widely variable and their use disagreeable and far from sanitary.

To use continuously the concentrated foods in the form of mineral fertilizers alone is not advisable, but when mixed with sufficient humus they become ideal.



One thing Alphano will produce in a surprisingly short time is a thick, vigorous sod, that will withstand a large amount of dry weather.

One of the chief values of humus is that it makes a perfect home and breeding place for the bacteria so necessary to fertility. These bacteria have a way of attacking the minerals locked up in the soil and converting them into a form available to the plant roots. Their activities also keep the soil open, so the air can work down through it, keeping it sweet.

Humus also has a way of absorbing four or five times its own weight in moisture, which acts like a big sponge in the soil. As a dry weather resistant, it is therefore decidedly valuable.

From all this it is evident, then, that the ideal soil builder would be one *rich in humus*, mixed with the essential plant foods and lively with the food liberating and nitrogen gathering bacteria.

Just such a soil builder is Alphano.

It is a perfectly balanced ration, prepared all ready for use. It is good for practically every growing thing.

This lawn was scattered with those old-fashioned flower beds once so much used. Last Spring, the owner, Mr. G. B. Richardson of Suffern, N. Y., planted them to grass, the soil being previously mixed liberally with Alphano. Four weeks later this photograph was taken. The only way you could detect the new grass was by its pronounced vigor and richness of color.

Natural Humus—An Explanation And A Caution

NATURAL Humus deposits are formed by the accumulation of countless years of decayed vegetable matter subjected to heat and moisture highly favorable for bacterial action. **Rich available deposits are of rare occurrence in nature.**

Deposits of peat and swamp muck, however, **are very common. They are made up of highly** acid organic matter, produced under conditions unfavorable to decay. **Such materials** should never be used as a soil builder. Their acidity and resistance to decay **make them entirely unfit for** plant food and *a distinct danger to plant life.*

At Alphano, N. J., there is a wonderful deposit of humus so free from acid and so rich in plant foods that when properly drained and deeply cultivated it at once produces abundant crops. This proves this particular humus to be ideal for soil-building purposes.

The humus we use as a basis for our Alphano comes from this remarkable deposit. It is first cultivated to prove its fertility and free it from all weed seeds, then it is electrically dug, beaten in fine pieces, aerated, sun-sweetened and dried and put in big piles, where it stays for months to further cure and multiply its soil bacteria before going through the concentrating and mixing process.

For your protection here is a comparison line-up of swamp humus and ALPHANO Humus.

SWAMP humus is generally acid and liable to be injurious to plant life.

ALPHANO Humus is sweet, and highly beneficial to plant life.

Swamp humus contains no soluble plant foods.

ALPHANO Humus contains all the soluble plant foods, such as nitrogen, phosphate and potash.

Swamp humus is harsh, wet and lumpy.

ALPHANO Humus is mild, dry and finely powdered, fit for broadcasting or using in drills.

Swamp humus is lacking in the good forms of soil-building and nitrogen gathering bacteria.

ALPHANO Humus contains all the good forms of legume and independent nitrogen gathering bacteria so essential to all soil fertility.

Swamp Humus contains approximately 1600 lbs. of water and only 400 lbs. of organic matter to every ton.

ALPHANO Humus contains approximately 1400 lbs. of actual humus matter to every ton. One ton of ALPHANO is equal to nearly four tons of swamp humus in its organic matter alone.

With these figures before you, you can see for yourself that swamp humus is worth only $2 per ton compared with ALPHANO at $8 per ton, when reckoned on the basis of the humus material alone.

ALPHANO contains so many more good things that there really is no comparison between the two.

It is a complete organic fertilizer and soil builder, carrying humus, lime, nitrogen, phosphate, potash and all the essential soil-building and nitrogen gathering bacteria.

Alphano—Its Strong Points

AFTER reading about Natural Humus on the opposite page, we can quickly explain to you exactly what Alphano is.

After the sun sweetened, aerated, Natural Humus has composted for several months, greatly multiplying its supply of soil building bacteria, we concentrate it in huge drying drums, requiring several tons of the Natural Humus to make one of the Alphano. Then it goes to the mixing room and liberal quantities of rich plant food nutrients are added to bring it up to a high fertilizing value.

This is followed by a liberal inoculation with our Alphano Inoculant, introducing in it all the essential nitrogen gathering bacteria that are so valuable for peas, beans, clover, alfalfa and such legume growths. See pages 20 and 21 for particulars of inoculation.

Now it is full fledged Alphano—a perfect soil builder, giving both immediate results and producing long enduring fertility.

Because of the abundance of humus it contains, Alphano will make a heavy soil lighter and a light soil heavier.

It breaks up compact, clayey soils, and binds together loose, sandy ones.

Because it absorbs so many times its own weight in moisture, it acts as a sort of internal reservoir, fortifying your lawn, flowers and vegetables against drying out.

Being rich in those foods that are *at once available* to the roots, results are immediate.

Being rich in those foods that are *slowly available*, its benefits continue for a surprisingly long time.

Being *so lively* with the soil building and nitrogen gathering bacteria, it attacks the locked up foods already in all soils, draws nitrogen direct from the air, and gathers it in nodules on the leguminous root growths, and in these ways further adds to and prolongs its benefits.

As satisfactory as are the results of Alphano the first season, its effects show even more the second. Alphano not only stimulates and furnishes food for your soil, but builds it up for years to come.

In addition to Alphano's building qualities, there are its distinct advantages of being *odorless*. You can use it *any time, anywhere*, without objection.

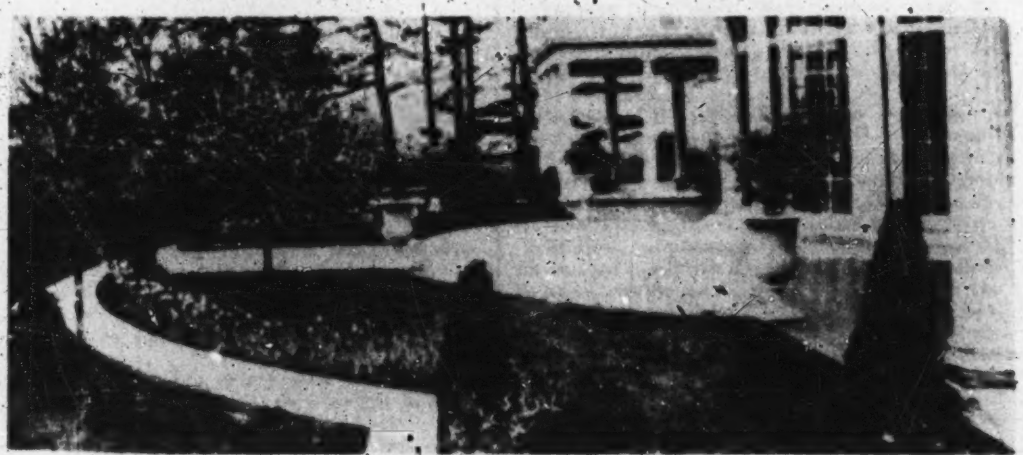
Its finely granulated form makes it easy to mix thoroughly with the soil, so that even the very finest of rootlets can be at once benefitted. It can be put on your lawn, raked in and *all* left there instead of being *raked off*, as is necessary with stable littered manures.

Being free from weed seeds it entirely overcomes one of the greatest objections to manures for lawns and gardens.

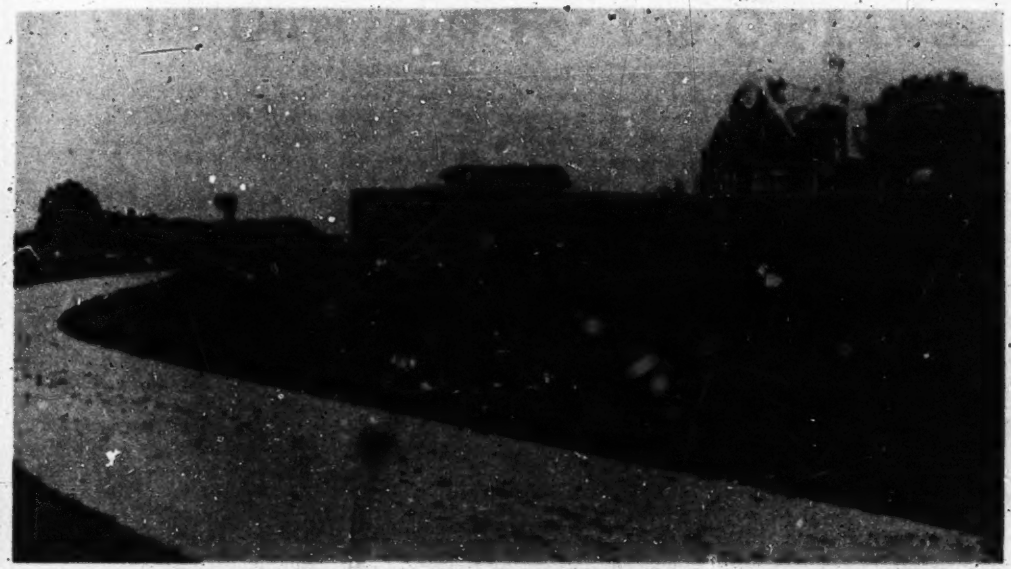
Being entirely free from plant diseases, maggots and other insect pests to which manure is heir, Alphano may be used with perfect safety on vegetable and flower gardens alike. You need never fear that Alphano will bring to your home, human or animal diseases such as are so commonly carried by manure; a few of which are typhoid, hoof and mouth disease, hog cholera, black rot, and such menaces.

Being so reasonable in price, you can use it freely all the season through.

For prices, see page 23.



It would be nothing short of a crime to litter this beautiful typical suburban residence with unsightly, unsanitary, fly-breeding stable litter. In its place Alphano is used, which is richer in plant foods, odorless and clean as sand.



Here is an interesting and conclusive bit of evidence of what Alphano will do for a terrace—the most difficult of all grassing problems. The top of the lawn was covered in the Spring with the usual heavy coat of stable manure. The terrace part with Alphano. The stable litter was of necessity soon *raked off*. The Alphano was *raked in* and became a direct part of the soil. The photo was taken three months later. The grass had been mown three days before. The difference in color and height of grass tells its own story of Alphano improvement.

What Alphano Does For Lawns Tennis Courts · Golf Links

IN the British Isles, where rains are frequent and "dampish" days come often, the grass forms in a compact, deep turf, having a rich green to the eye, and a delightful springiness to the foot.

Nowhere do you see finer lawns. To duplicate them in this country has generally been considered a great expense, and therefore indulged in only by the fortunate few.

The results secured, however, in the last ten years with Alphano prove that if climatic conditions are not so favorable as in England, practically the same results can be secured at a low cost, by building up such soil conditions as will be most fortunate for not only thick mats of "cushiony" *surface* roots, but induce a *deep*, vigorous root growth as well.

Of first importance, then, is to put into the soil something that will act like a hungry sponge, which, filling full of moisture, gradually liberates it as needed.

Alphano, with its rich humus base, absorbs from four to five times its own weight in moisture, meeting that requirement perfectly.

Its quickly available foods are an immediate stimulant to the top roots, while the slowly liberated foods, becoming a direct part of the soil, are in part carried deeper, inducing the larger roots to readily follow. This deep root growth in itself is a protection against dry weather.

Alphano overcomes that compact, unyiving condition of clayey soils, inducing a springiness. On sandy soils it binds the particles together, giving it a body and stability for the roots to hold themselves in.

Because of the offensiveness and unsightliness of stable manures, lawns that otherwise should each year be enriched are often neglected for two or three seasons, and so the soil foods are spent. The frequent close cutting also rapidly exhausts the soil, making life a weary grind for the roots.

Odorless, weed-seedless Alphano, because of its ease of application and freedom from offensiveness, can be used at any time, and with the first cutting of grass be entirely out of sight. But it's there, just the same, busy root building.

We were recently fortunate in securing the services of an intensely practical man on lawns, to write for us a book, entitled "Lawns and Golf Greens, Their Care and Fare." If you want to go into the question thoroughly, drop us a line and we will gladly send you the book with our compliments.

Brief Directions For Use On Lawns

They are brief because this question of lawn making and repairing, for example, is so big that to do it justice needs a book all to itself, so we only touch on it here. But our book on "Lawns and Golf Greens Their Care and Fare," you are welcome to. We suggest your sending for it. It covers the ground concisely and completely.

Old Lawns

DECIDEDLY contrary to general impression, it is not necessary to tear up an old lawn in order to get a satisfactory result.

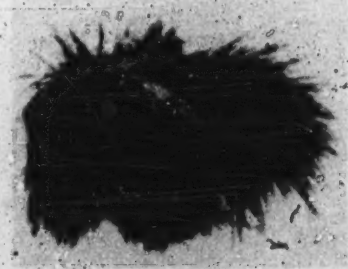
For every 1000 square feet of fairly good soil use at least two 100-lb. bags of Alphano. For poor soil five bags.

Rake or disk harrow every part of the lawn over deeply. Thoroughly spread on the Alphano. Rake or harrow in well. Mix 8 ounces of English rye grass with every pound of your seed mixture to act as a nurse. It will come up quickly, grow rapidly and protect the finer grass from the sun and when closely cut makes a satisfactory lawn in itself.

Use at least 2 to 3 lbs. of seed to each 1000 square feet for re-seeding. Or 3 to 5 lbs. in making new lawns.

Sow your seed first lengthwise and then crosswise to get an even distribution. Now roll it. If planted in Spring, grass will show in 10 to 15 days. In the Fall, after 5 to 10 days.

This 4½-inch deep sod was made in six weeks from the sowing of the seed. The soil was a regular sterile white sea sand, mixed with one-half Alphano.



Bottom view of the piece above. Notice the thick mass of fine rootlets, indicating the height of vigor and drought-resisting power.

A light top dressing of two-thirds soil and one-third Alphano will be highly beneficial after six weeks.

New Lawns

IF you want to go right to the bottom and make your old lawn entirely over, then deep spade it, or plough where possible. Do it so well that all sods will be *entirely buried*. Follow with raking or harrowing. Spread on Alphano at the rate of 800 lbs. to every 1000 square feet for ordinary enrichment, or up to 400 lbs. for long enduring fertility, with little or no after treatment. Rake in, seed, roll.

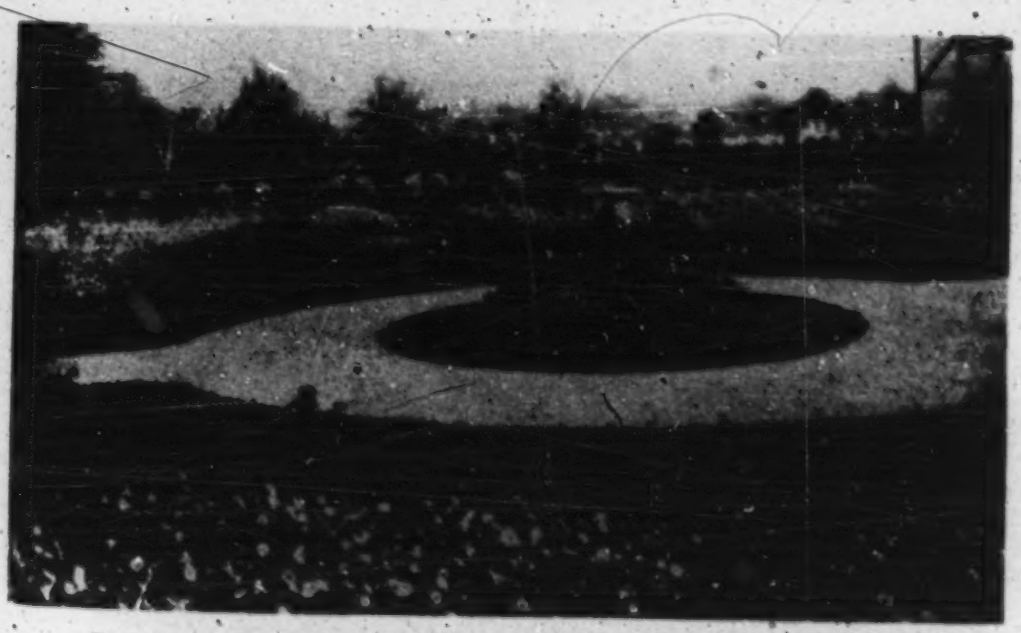
If your lawn site is one newly graded the chances are the soil is mostly sub-soil and practically devoid of humus. In which case after smoothing up you should use at least 200 lbs. of Alphano to every 1000 square feet, then follow the same as above.

In six weeks make a mixture of two-thirds soil and one-third Alphano and spread on lightly, using say 100 lbs. of the mixture to every 1000 square feet.

Spread the Alphano on with a shovel, then rake it over evenly, followed by *surely raking it in thoroughly.*

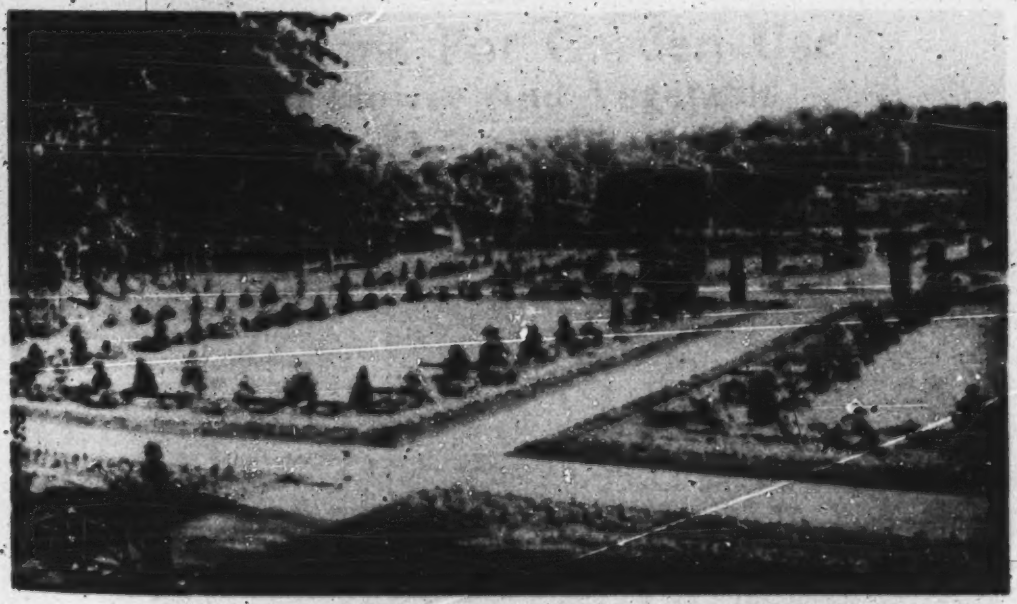
If you fail to rake this top dressing thoroughly into the soil, results will not be so good; in which case blame yourself—not Alphano.

Early in June this grass was thin and dotted with bare spots. It is approximately 60 feet long and 30 feet wide. Eight bags of Alphano were used. On the 15th of July, when this photograph was taken, it was fast becoming a perfect lawn.



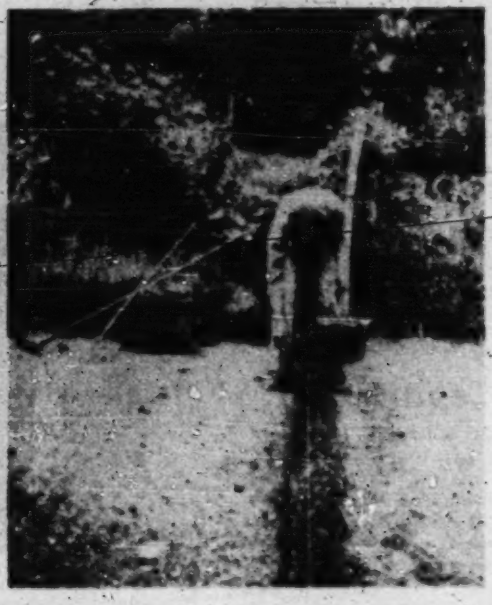
The light, sandy, Long Island garden soil on Mrs. A. M. Booth's grounds at Great Neck, L. I., was converted into a soil of ideal texture and wonderful productiveness by a season's use of Alphano.

Up in the mountains near Tuxedo, N. Y., where the soil is thin and the stones many; this extensive formal garden was made possible by the liberal use of Alphanu. The grass walks of this garden must certainly vie with those of England's famed "300-year-old turf." Name of owner gladly given on request.



Starting with a boggy swale, the City of New York had this piece of ground filled in with all sorts of city refuse and topped with a mere two feet of good field soil, lacking in humus. Tons of Alphanu were finally used and this Van Cortlandt Park, beauty spot burst into greenery and bloom. Samuel Parsons was the landscape architect.

Showing the raking in of Alphamo, after your garden has been spaded up and smoothed off. Use 300 pounds or more for every 1000 square feet.



Putting the Alphamo in the row before sowing the seed. It's always a good plan to do this. It insures the first roots getting plenty of nourishment, just *when they most need it.*

Directions For Garden Use
Both Flower And Vegetable

THERE are at least four ways you can garden happily and effectively with Alphamo:

1. Spread it on the soil and spade in, just like is done with stable manures.

2. First spade up, smooth off and then spread on the Alphamo and rake it in well. See illustration.

3. Sow in the row or hill, mixing it with the soil and then plant your seed. See illustration.

4. Dig it around the plants once or twice during the season to give them a good, vigorous boost.

If your garden soil is in badly impoverished condition and you want to surely have a model garden the very *first season*, you could well adopt all four ways.

If in fair shape, 2, 3 and 4.

If a good, friable, rich soil, then 2 and 3.

If you have at times been a bit boastful of your garden prowess, after using Alphamo you will surely be in a position to make your neighbors still more envious.

If your garden is a large one, and you don't feel like spending enough the first year to entirely rebuild the soil, then follow numbers 3 and 4.

Being odorless and in granulated form, Alphamo is an agreeable, sanitary solution to all your garden enriching problems.

The second year after you have adopted Alphamo as your garden helper you will have even better results than the first.

The busy millions of bacteria will then have had a better chance to get in their work and the Alphamo, will also by your season's tilling be more evenly mixed with the soil.

After Alphamo has once brought your soil up to a point of high fertility, it will be an easy matter to keep it there.

For Peas and Beans

Peas and Beans being legumes, the nitrogen gathering bacteria with which we inoculate Alphano will form nitrogen filled nodules on the roots, greatly increasing the rapidity of growth and quantity of yield.

These root nodules left to decay in the soil, at the end of the season, contribute liberal amounts of nitrogen for the benefit of any next season's crop.

For Sweet Peas

If you have had disappointments with Sweet Peas, the results from using Alphano will make your heart glad. Sow it freely in the drill.

Don't make the mistake of hoeing the soil up around the stems. It is liable to cause stem rot. Frequent loosening up of the soil, however, is essential.

The surprising thing about this celery is that it grew on a heavy clay upland soil that the year before refused to grow beans more than four inches high. When such soil can be induced the next season to grow celery 3½ feet from root end to top tips, it shows rather conclusively what Alphano will do if given a chance. Three inches were spread on and mixed in. Name of owner on application.

Setting Out Plants

Many of the uncertainties of plant setting out are overcome by the use of Alphano.

Mix it well into the soil so it will surely come in direct contact with the rootlets. Its readily available foods will promptly stimulate growth, while its property of retaining moisture cuts down to a minimum, weak backs caused by wiltings.

Boostings

Along the middle of Summer when all growth has drawn so on the soil foods, your plants will welcome the boost Alphano will give them if dug around their roots.

Being in dry, granulated form, you can do it easily and quickly. Being odorless, absolutely sanitary and clean as sand, its use is free from all objections.

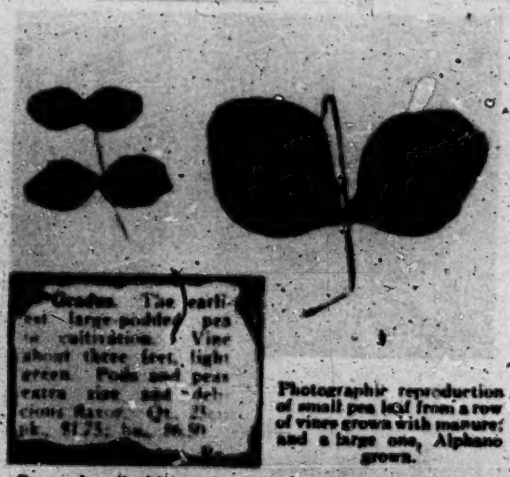
Started Aug. 4, digging Alphano around the plants. Photo taken in the early spring.

Same view of the same garden, even to the same enthusiast. Photo taken last of July, same year.

Results With Peas

ON June sixth of last year, Mr. B. T. Joyce of West Haven, Conn., wrote us the following:

"Just to let you know what Alphano Humus is good for in my garden. I used four bags on seventeen rows of Peas, each row 135 feet long. I planted them five weeks later than some of my neighbors. I have vines 4 feet high and they are only supposed to grow 3 feet high. Enclosed find a clipping from the seed catalog and two samples of leaves, the large leaves from my garden and the small from a neighbor's where horse manure was used. I also have Peas as large if not larger than other Peas planted five weeks earlier. A picture would be the only way I could prove it to you, but if you examine the leaves it will help to convince you." (Signed) B. T. Boyce.



Photographic reproduction of small pea leaf from a row of vines grown with manure; and a large one, Alphano grown.

Pea as described in catalog.

Three Times The Quantity Of Potatoes

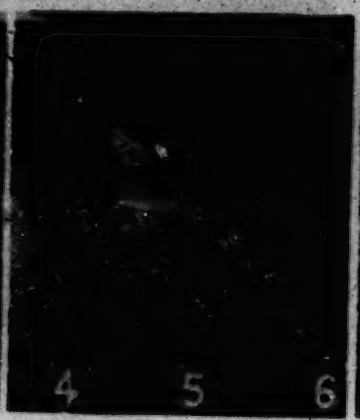
THE wording of the declaration below is taken from the affidavit of Mr. C. W. Baines of Newport News, Va., dated July 8, 1915, made before H. H. Noblus, notary public and now on file at our office.

Newport News, Va., July 8th, 1915.

"I, C. W. Baines, hereby certify that the below is an actual photograph of six (6) rows of Irish Potatoes which I raised on my trial grounds. The rows numbered 4, 5 and 6 were treated with commercial fertilizer ($45 per ton), while those numbered 1, 2 and 3 were treated with a like amount (not value) of Alphano Humus."

To secure that freshly succulence so delicious in lettuce perfection, it must be grown quickly. That's one of the things Alphano makes possible. It has the properties of inducing a quick growth, as well as a solid one. For coldframe use, it is ideal.

Rows 1, 2 and 3 were given same amount of Alphano at $12 a ton, as rows 4, 5 and 6 with chemical fertilizer at $45 a ton.



Rows 4, 5 and 6, in spite of using a fertilizer nearly four times the cost of Alphano, made poor growth.

Potatoes in left hand pile came from row No. 2. The right hand one from row No. 4.

This suburbanite had come to the conclusion that he never could have a successful flower garden with his lumpy, pasty soil. One year he gave it up altogether. Year before last he tried it again by using Alphano. From the results this photograph shows, it is not to be wondered that he has become a real gardening lover. Name gladly given on request.

We have yet to find the flower that does not thrive vigorously with the use of Alphano in proper soil mixtures. On an extremely light, sandy Long Island soil, it each year produces this continuously beautiful garden. It seems to bloom earlier and last longer than other nearby gardens.

So far, there is nothing we know of that will prevent leaf rust on Hollyhocks. It is a noticeable fact, however, that good, vigorous, quick growing plants are least affected. Dig Alphano liberally around the roots and give them a try. Results will surprise you.

For Your Shrubs and Trees

DON'T slight your shrubs and trees when it comes to enriching your lawn and gardens. It's surprising the response you will get from them for such "encouragement."

Shrubs that have been growing slowly and blooming indifferently will take on a new lease. Before the blooming time is a good time to use Alphapo as it increases their size. A month or so after, is also a good time as the shrubs are then often exhausted by the blooming. May and June are the months when both trees and shrubs make their growth. The rest of the Summer the growth then made is hardening up to withstand the Winter.

That the roots should have ample food during this period of greatest growth is obvious. Another thing to remember is that many of the shrubs bloom next season *on only the new growth made this.* Abundant growth this season means abundant blooms next.

The result of Alphapo used on Golden Privet. The roots at the left of the line were the original ones. Those at the right are *all new.* Their growth was induced in one season, entirely by the use of Alphapo. Vigorous root growth means flourishing tops. Most shrubs are great feeders. Slight their food and your results will be proportionate.

Don't put a scanty shake of Alphapo around your shrubs and then dig it in gingerly. First make the ground nice and mellow all around the roots for a goodly distance, and then dig plenty of Alphapo well in. Once or twice during the season, loosen the earth up. Given even half a chance, shrubs respond wonderfully.

Tree Planting and Feeding

When planting your shrubs, start right by using Alphano with water, make a semi-liquid mixture of two-thirds soil and one-third Alphano and stick the roots into it, or plaster it thoroughly on them. It moistens them and furnishes an immediately available food to bridge over the time until they get a grip on the soil.

Spread the roots out well, as all directions, make sure the tree stand straight and then fill in with dirt mixed with at least a third of Alphano. Be sure to pack tightly immediately around the roots, so they can quickly get a hold on the soil fresh. Loosen the top soil at least twice during the season. Each year top dress with Alphano and dig in well. If you want maximum results this is the way to get them.

Some think that because evergreens generally come packed with a ball of their native earth around their roots they need no enriching the first season. Transplanting at best is always an ordeal for any tree. A quick recovery means quick growth. Make their food rich and easy to reach. Plant them with soil mixed with at least one-third Alphano.

The fine food-absorbing rootlets of trees are at the ends of the larger roots. To dig Alphano around close to the trunk of a tree helps a little; but to secure the greatest benefit, it is necessary to go out as far as the branches extend and then dig a circle trench around the tree; fill in with soil mixed with one-third Alphano. Do it thoroughly this way and then it's done for several years to come.

Rhododendrons on a famous New Jersey estate. Alphano was used by the carload on the lawns and around the flowers, shrubs and trees of this extensive estate.

Ideal For Rhododendrons

IN their native habitat, Rhododendrons are found growing in rich, humus-laden soil formed by decayed forest leaves.

The nearer then, you come to duplicating such soil, the finer will be your plants. Alphano's base being just such a rich, thoroughly decayed humus, it makes an ideal base for Rhododendrons when mixed with from one-half to two-thirds soil. In the Fall, bank them all around with leaves at least two feet deep, laying sticks or branches on them to prevent their being blown away. In the early Spring remove the leaves, mulch with Alphano, gently mix into the soil. The root mass is near the surface, so have a care not to dig too vigorously. See that they have plenty of water. They are no relatives of the Cacti family.

Do not lose sight of the fact that the Rhododendron buds for next Spring's blooms are formed at the end of the new May-June branch growth. It is a critical period for them. See that the roots have *ample food* and moisture. Mulch heavily with Alphano.

For Potted Plants And Window Boxes

HOW to either secure or make a proper soil mixture for window boxes and potted plants is an ever recurrent problem with many. Even if you are so fortunate as to start with a fairly rich soil it soon loses a large percentage of its foods by leaching away with the constant waterings always necessary.

Being confined in such limited spaces, the soil must be of an open, friable nature, to secure a free circulation of air. It must have body enough to hold the moisture but not so heavy as to keep the roots waterlogged. Almost any soil, heavy or light, can be converted into an admirable one for the purpose by a mixture of Alphano. For good garden soils one-quarter Alphano is sufficient. For poor soils, one third.

In addition to mixing the soil with Alphano, it is well to dig it some, once or twice during the season. Being odorless you can do this without objection at any time.

Straggly, leggy plants have no place in your window boxes. You want them stocky, and healthy *all the way through*. Soil mixed with one-third Alphano will give splendid results all Summer. A top dressing of it dug into the soil once or twice during the season will also be found decidedly beneficial.

The problem on this estate was to produce in two years, results that would give the effect of being established for fifty years. Extensive grading was done, big trees moved in, and large shrubs planted freely. To insure an immediate and lasting fertility, Alphano was used liberally. Here is a pile waiting distribution. Name of estate on request.

For Resoiling Purposes

IN grading and changing the conformation of grounds the question of fertile top soil is a serious one. The average depth of top soil is seldom over a foot. This soil contains the essential humus which plant life demands for its thriving.

The sub-soil is lacking in such humus. This being so, you can appreciate more than ever why it becomes such a difficult problem to secure satisfactory growing results on a piece of ground the grading of which has required the removal of soil from some portions and filling it in at others.

It is in just such cases that Alphano is of great value. By its use you can greatly increase the fertility of an already fertile soil and quickly make sterile sub-soils satisfactorily fertile.

Even seashore sand can be made productive when mixed with Alphano. The above picture, taken on a Long Island estate, shows one of several piles of Alphano amounting to 1700 tons, which was used in an extensive resoiling operation.

Before placing this 1700-ton order the owners had first convinced and re-convinced themselves of the merits of Alphano by giving it trying tests in locations where they could keep continual watch of the results.

One ton of Alphano contains nearly two yards of pure humus, or an amount equal to what is generally found in 40 to 50 yards of such soil as is usually sold for from $2 to $3 a yard.

It is self-evident that Alphano at $4.50 to $5 a yard is worth at least twenty times as much as ordinary soil in humus alone, not to mention the rich plant foods and beneficial soil bacteria it contains. An absolutely barren, sandy waste at Glen Cove, L. I., was in a season converted into a garden spot where vegetation now blossoms and flourishes like the green bay tree.

The Alphano for this estate was shipped in cars to Hoboken, N. J. and taken in barges up Long Island Sound to Glen Cove.

Use In The Greenhouse

THE intensely intensive methods employed in the successful greenhouse growing of flowers, fruits and vegetables require a soil of high fertility. The combination of artificial heat and conservation of the sun's rays by the glass causes an atmosphere which quickly dries out the soils. The problem of watering is one requiring great judgment. Too little causes the roots to struggle for moisture, retarding top growth; too much sours the soil, checking root growth.

As the season advances, especially with "strong feeding" plants, top dressings of either barnyard manures or bone meal were, before the advent of Alphano, necessary. Both of these are of offensive odor. The manure is obnoxiously unsightly, making the visiting of one's glass enclosed garden at such times anything but a pleasure.

On eight joints of these 3ft tall high Alphano fed tomato vines there are fruit clusters. One joint is in bloom and another forming buds. Nothing has ever approached Alphano in this man's experience with his former use of manure or other fertilizers. Name of grower on application.

If you are one of the fortunate flower lovers who enjoy showing your devotion to your flower friends by working among them yourself, such objections as these greatly discount your pleasure.

Alphano in proportion of one to three parts of soil makes a greenhouse mixture that is entirely free from odor. As an odorless top dressing it is highly resultful and without the objection of unsightliness.

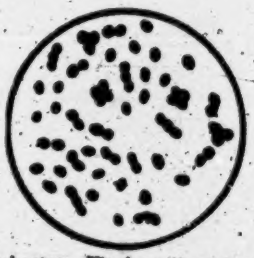
Being capable of absorbing many times its own weight in moisture, frequent watering is not necessary—in fact it is to be guarded against.

For making a potting mixture the gardeners tell us it is just about ideal.

Order some and try it out on one of your benches.

To be able to go into your greenhouse at all times and find it free from obnoxious soil enrichment odors, is one of the desirable things in favor of Alphano.

Photographic reproduction of a colony of Radicola, one of the busiest nitrogen-gathering bacteria, magnified one million times.



Azotobacter. The bacterium that fixes atmospheric nitrogen.

Root of crimson clover, showing the nitrogen nobs or nodules. They are filled with thousands and thousands of nitrogen-gathering bacteria.

Soil Inoculation and Something About Alphano Inoculant

Inoculated crimson clover grown with rye, which acts as a nurse crop.

ALL fertile soils contain what might be called natural bacteria that in their activities convert the soil minerals into forms readily available to plant life. They also do an important part in helping to keep the soils open so the air freely circulates through them. So minute are they that thousands, can lodge on the head of a pin.

Twenty or so years ago it was discovered that if soils in which clover had grown freely were scattered lightly over ground that failed to yield a satisfactory crop the production would be greatly increased.

Microscopic investigation and bacteriological tests proved that the little nobs or nodules on the roots of all Clover and other legumes are filled with nitrogen gathered from the air by certain nitrogen converting bacteria with which they were particularly lively. The abundant presence of these nodules always went hand in hand with large crops.

It was also noticeable that when the roots of clover, cow peas, vetch and like legumes were ploughed under the ground's fertility was greatly increased for subsequent crops of whatever kind.

Then followed the discovery that there were several legumes that had their own private bacteria, as it were, that would work only for them. Following this the various bacteria were isolated and by cultural processes, multiplied and sold for inoculating seed before being sown. When handled consistently the results were satisfactory. But the highly artificial medium in which the bacteria were held and shipped made their existence more or less uncertain and results from their use variable.

After a long series of experiments conducted by a well-known bacteriologist, of one of our foremost Agricultural Colleges; he discovered that our Alphano was an ideal home for all the legume bacteria. In the Alphano they multiplied with rapidity, gained great vigor and retained their vitality under conditions utterly impossible in the other mediums.

He also made the phenomenal discovery that the bacteria of all the legumes could be grown together in Alphano. The astounding fact also developed that this inoculated Alphano can then be used to inoculate the soil for any and all legumes. No longer is it necessary to buy a separate inoculant for each legume.

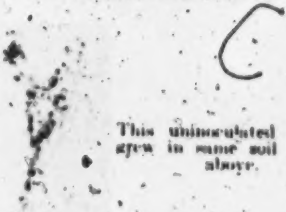
It is this all-in-one material, called Alphano Inoculant, with which we inoculate our regular prepared Alphano.

We are now offering it for sale in 2-lb. cans, sufficient for inoculating one acre, and in 20-lb. cans, enough for ten acres.

For full particulars send for the Alphano Inoculant Booklet.

For prices see over the leaf to page 23.

Inoculated soil grew this Alfalfa



This uninoculated Alfalfa grew in same soil as the above.

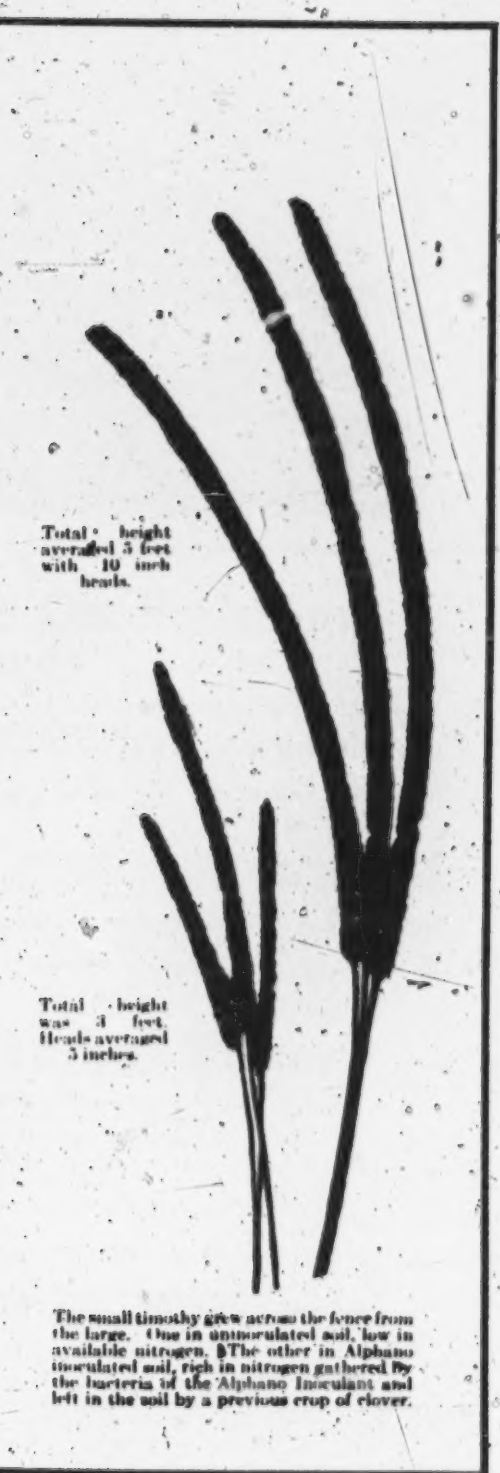
For Composting Use Alphano

THE mixing of Alphano with your compost heap will not only give a highly valuable increase in plant foods and humus, but bring to it countless billions of soil food converting; and nitrogen gathering bacteria, that will inoculate the entire compost heap and in turn any soil it is used on.

It is a sure method of increasing at a very low initial cost the quantity and fertilizing value of your compost.

Alphano may also be used to great advantage as an absorbent under the stable bedding, in the drops behind the cattle and in the poultry house. As an absorbent of liquid excreta it has several times the value of phosphate floats.

For inoculating purposes only, sprinkle the heap or loads as taken out with soil mixed with the Alphano Inoculant.



Total height averaged 5 feet with 10 inch heads.

Total height was 3 feet. Heads averaged 5 inches.

The small timothy grew across the fence from the large. One in uninoculated soil, low in available nitrogen. The other in Alphano inoculated soil, rich in nitrogen gathered by the bacteria of the Alphano Inoculant and left in the soil by a previous crop of clover.

This Alphano-ized garden and lawn make you think of the wonderful results for which England is so famed.

Free Expert Advice On Soils and Grass Seeds

MUCH time and no little money are wasted simply because people persist in ignoring the simple rules of cause and effect when it comes to questions about soils.

They will, for example, seed and re-seed acid soils and complain bitterly because they can't produce a satisfactory sod.

There is no use bumping up against Nature's rules, because she *always* does her return bumping vigorously.

Indulge her, however, and your reward is ample.

If you will fill out one of our soil question blanks and mail it to us, our experts will gladly advise you concerning the treatment and handling of any of your soil to secure its highest production fertility.

Based on this information, we can then also intelligently suggest the grass seed best suited to both your soil and climatic conditions.

If the soil question blank enclosed in this book has happened to slip out, let us know and we will gladly send you another.

Send For Soil and Humus Tester

FOR your own use you should surely have one of our Alphano Soil Testers, so you can with surprisingly little trouble quickly find out for yourself whether your soil is acid or alkaline and what its humus content is.

Along with the tester we will send you a guide that tells you exactly what to do under varying soil acidity and humus conditions.

An acid soil is just as detrimental to the health of most plant life as an acid stomach is to you.

Lime is the universal sweetener for soil. But the quantity used must, of course, be governed by the amount of acidity to be corrected.

This, the Alphano Soil Tester will tell you.

We will gladly send it to you, postpaid for $1, or send it with our compliments with an order for 5 bags or more of Alphano.

Prices

Alphano Humus

5 Bags, 100 lbs. each	$5
By the ton, in bags	$12 a ton
By the car, in bags	$10 a ton
In bulk, by car or barge load	$8 a ton

These prices are for delivery on board cars at Alphano, N. J.

Alphano Inoculant

2-lb. tins, enough for one acre	$1
20-lb. tins, enough for ten acres	$5

We prepay cost of delivery from our factory to all points east of the Mississippi when cash accompanies order.

Terms

CHECK, draft or money order should accompany all orders for less than two tons. Satisfactory references will, however, have consideration on orders for anything above two tons.

Method of Shipment

ALPHANO is usually shipped in bulk in carload lots. In less than carload lots, it is forwarded in bags. Special freight rates have been made by the various railroads from Alphano, N. J., to all principal shipping points. When transportation by water is practicable, barge-loads are delivered alongside nearest dock to customer's estate or beached upon the property itself if shore and weather permit. This effects considerable saving in freight charges. *Alphano Inoculant* will be sent delivery prepaid, east of the Mississippi when remittance for prices given in this book accompanies order.

Guaranteed Analysis of Alphano Humus

Chemical Element	Per Cent
*Nitrogen	1.25 to 2.50
Ammonia	1.50 to 3.00
Available Phosphoric Acid	.50 to 1.00
Potash	.50 to 1.00

Balance, Humus Vitalizers, Lime and Moisture.

*Humus Nitrogen.

These are our huge drying drums where the humus, after being aerated and composted is slowly concentrated with a mild heat which drives out 75 per cent of the moisture while still preserving its natural rich bacterial life

You Are Cordially Invited To Visit Our Works At Alphano, N. J.

WE want you to visit our works. They are located in the beautiful Pequest Valley, a scant six miles' auto ride from Hackettstown, N. J. Or you can reach it on the Lackawanna Railroad by way of Andover Junction and the Lehigh and Hudson River road. Also by taking the Erie to Greycourt and changing to the above road.

We want you to see the rare Alphano deposit of humus, with some areas still covered by growth primeval.

Glimpse along a stretch of our 10-mile private railroad. So luxuriant and abundant is the vegetation you almost feel you are in the midst of some tropical jungle

We want you to see the interesting process from start to finish of converting the humus into the wonder-working soil-builder, Alphano.

We want you to see the huge composting pile.

And if you are a little adventurous; you will surely want to go chug-chugging over to the western deposit in the engine of our own private railroad.

You will find such a visit interestingly instructive. You will leave, feeling amply compensated.

January, 1923 Bulletin 377

Results of Seed and Legume Inoculant Inspection for 1922

NEW JERSEY

AGRICULTURAL

Experiment Stations

Department of Seed Analysis

New Brunswick, N. J.

DEPTS. EX. 19 IDEN.

PART 2. LEGUME INOCULANT INSPECTION

Introduction[6]

In accordance with the law governing the sale of legume inoculants, passed by the New Jersey State Legislature in 1919, the market has been inspected and samples collected for official analysis. Considerable time has been spent in a successful attempt to remove from the market all old and out-of-date stock and in collecting the official samples. The samples as collected have been forwarded to the Experiment Station for purposes of official tests which have been conducted in accordance with the method outlined below. The results of these tests have been placed in Table 61.

The Legume Inoculant

The law defines the term legume inoculant as any material which contains the nitrogen-fixing organism, *Bacillus radicicola*, and which is sold, offered or exposed for sale for the purpose of conveying organisms of *Bacillus radicicola*. This law states that all preparations sold for this purpose shall bear a label giving the following information:

1. The brand name.
2. The kind or kinds of legume crops for which the contents may be used.
3. The month and year in which the inoculant was prepared.
4. Probable date beyond which inoculant would be of doubtful value.
5. Quantity of material in terms of amount of seed or acreage for which the contents may be used.
6. Name and address of manufacturer.

It is required, furthermore, that all persons or companies wishing to sell legume inoculants shall file with the Experiment Station annually a statement of the brand or brands that they wish to sell and the name and address of the manufacturers.

The penalty for violation of the law is a fine of twenty-five ($25.00) to one hundred dollars ($100.00) for each offense.

In addition to this regular control by means of official analysis the law provides that any citizen of the State of New Jersey may obtain, free of charge, a report on the examination and analysis of any sample or samples of legume inoculants. To secure these services it is only necessary to send the proper samples to the New Jersey Agricultural Experiment Station, transportation charges prepaid. These analyses are classed as unofficial. The results of tests of unofficial samples are published in the Forty-third Annual Report of the Experiment Station, 1921.

Analysis of Official Samples

The stock of the registered dealers (see table 60) has been inspected and found to be in good condition (see tables 61 and 62).

In table 62 are listed the test numbers of all cultures that gave either poor inoculation or failed to meet the purpose of the law in regard to their

[6]The inspection and analysis of legume inoculants has been conducted by Alfred T. Perkins, M.Sc.

labels. The exact reason for listing these cultures is indicated in table 61. However, it should be noted that the cultures, 246, 255 and 264, labelled "peas, beans and sweet peas," gave good inoculation for garden peas, garden beans, lima beans and sweet peas but failed in the inoculation of soy beans and cow peas, which require a different specific organism. It should also be brought to attention that cultures 255, 261, 262, 263 represented a stock of material manufactured in 1922 and withdrawn from the market immediately after inspection.

The methods in testing the cultures are in brief as follows: bacterial counts and purity tests are made and recorded. The cultures are diluted to the proper degree, applied to sterilized seeds, and planted in fertilized and sterilized sand. The pots in which the inoculation tests are conducted are kept in the greenhouse, where moisture and temperature can be controlled. Purity tests are not reported, since an impure culture is considered to be of the same value as a pure culture containing the same number of radicicola organisms.

The inoculation tests have been recorded as "O. K." which indicates good inoculation, as "Fair" or "Poor," which indicates a positive test, and as "None," which indicates no inoculation.

Attention is called to table 63, in which are grouped the several host plants inoculated by the same organism. It may be considered that a culture inoculating one plant of a group will inoculate the other plants in the same group.

As an aid in studying the figures listed in table 61 near the heading, "Number of organisms per acre," table 64 has been inserted, which gives the number of seeds of the leguminous plants commonly sown per acre.

Summary and Conclusions

The tables show that the market of legume inoculants is in good condition. During the year the stock of improperly labelled material has been reduced to a minimum. Since the cultures on the market all bear a date of manufacture and a guarantee of the time for which the cultures will remain viable, little more can be done except to run tests which will indicate whether or not the guarantee of the companies are fulfilled. The vast majority of such tests in the past have given satisfactory results.

Table 65 gives a summary of the cultures tested and table 66 the number of dealers in legume inoculants registered in each county of the state. From these tables it may be seen that the principal inoculants sold are those of the Earp Thomas Corporation and of the H. K. Mulford Co., while the principal area of their use is in south New Jersey.

OFFICIAL LIST OF REGISTERED DEALERS—TABLE 60

Dealer's Name	Address	Brands of Inoculants Registered
Andrewes Drug Store	Broadway, Salem	Mulford Culture
E. H. Berry	Toms River	
Blew & Blew	Bridgeton	Mulford Culture
Burlington County Supply & Produce Co.	Mt. Holly	Farmogerm
S. E. Burr Hdwe. Co.	Bordentown	
J. S. Collins & Son, Inc.	Moorestown	Mulford Culture
Geo. De Baum & Son	253 Main St., Hackensack	Farmogerm
Dixon & Fogg	Broadway, Salem	Mulford Culture
Farmer's Seed Store	38 S. Pearl St., Bridgeton	Mulford Culture
Alexander Forbes & Co.	115 Mulberry St., Newark	Mulford Culture Farmogerm
Ralph B. Gable	Landis Ave., Vineland	Mulford Culture
Hart & Co.	Paterson	Farmogerm
John Molitor	Swedesboro	Mulford Culture
Monmouth County Farmers' Exchange	Freehold	Farmogerm
Muzzy Bros.	143 Main St., Paterson	Farmogerm
Perrine & Buckelew	Jamesburg	Alphano Ino.
Martin C. Ribsam	Trenton	Mulford Culture Farmogerm
Jacob Rubinoff	Vineland	Alphano Ino.
Sharp, Alwater & Haver	Flemington	Farmogerm
H. K. Shoemaker	Swedesboro	Mulford Culture
So. Jersey Farmers' Exchange	Bridgeton	Alphano Ino.
Swedesboro Supply Co.	Swedesboro	Mulford Culture
Taylor Bros.	Front and Market Sts., Camden	Mulford Culture
A. Tepperman	Long Branch	
Tuohy & Lounspury	Market St., Salem	Mulford Culture
Geo. W. Weber	301 High St., Millville	Mulford Culture
Westrobac Sales Agency & Agents	Orange	Westrobac
Geo. H. Whipple & Son	63 Broad St., Bridgeton	Mulford Culture
Jacob R. Wyckoff	Princeton Junction	Farmogerm
W. H. H. Wyckoff Co.	33-35 Middaugh St., Somerville	Farmogerm

RESULTS OF OFFICIAL ANALYSES—TABLE 61

Test Number	Town Retail Vendor Wholesale Vendor	Crops and Inoculation		Organisms per package (Millions)	Organisms per acre (Millions)	Date of preparation Guarantee Date of test
	TRENTON					
236	Martin C. Ribsan Earp-Thomas Co.	Vetches	O. K.	735 1 acre	735	8-21-20 5 years 9-21-21
237	Martin C. Ribsam Mulford Co.	Soy Beans	O. K.	1,400 1 acre	1,400	5-19-21 1 year 9-19-22
281	Martin Ribsam Earp-Thomas Co.	Red Clover	O. K.	12,760 3 acres	4,253	1-10-22 5 years 5-9-22
282	Martin Ribsam Mulford Co.	Red Clover	O. K.	304,500 5 acres	61,000	4-1-22 1 year 5-9-22
277	Martin Ribsam Earp-Thomas Co.	Red Clover	O. K.	6,597 1 acre	6,597	3-8-22 1 year 5-9-22
278	Satterthwaite's Seed Store Earp-Thomas Co.	Red Clover	O. K.	86 3 acres	29	3-9-22 5 years 5-20-22
279	Satterthwaite's Seed Store Earp-Thomas Co.	Alfalfa Swt. Clover	O. K.	1,100 3 acres	366	1-10-22 5 years 5-9-22
280	Martin Ribsam	Garden Beans	None	327 Garden Size		3-4-22 1 year 5-9-22

RESULTS OF OFFICIAL ANALYSES—TABLE 61—Continued

Test Number	Town Retail Vendor Wholesale Vendor Commercial Name	Crops and Inoculation	Organisms per package (Millions)	Organisms per acre (Millions)	Date of preparation Guarantee Date of test
	VINELAND				
274	Shenberg & Rubinoff Alphano Humas Co.	*All Crops* Soy Beans O. K. Garden Beans O. K. Alsike O. K. Alfalfa O. K. Vetch O. K. Lupines O. K.	1,408 1 acre	1,408	3-8-22 1 year 5-20-22
275	Ralph Gable Mulford Co.	Alfalfa O. K.	4,745 5 acres	949	4-24-22 1 year 5-9-20
276	Ralph Gable Mulford Co.	Soy Beans O. K.	2,934 5 acres	587	4-24-22 1 year 5-20-22
240	Shenberg & Rubinoff Alphano Humas Co.	*All Crops* Cow Peas O. K. Soy Beans O. K. Garden Beans O. K. Alsike O. K. Alfalfa O. K. Vetch O. K. Lupines O. K.	8 1 acre	8	8-13-22 1 year 9-13-21
	MOUNT HOLLY				
241	Burlington County Supply & Produce Co. Earp-Thomas Co.	Vetches O. K.	113 1 acre	113	Not given 5 years 9-14-21
242	Burlington County Supply & Produce Co. Earp-Thomas Co.	Alfalfa O. K.	185 1 acre	185	8-16-21 5 years 9-14-21
	FLEMINGTON				
243	W. H. H. Wyckoff Earp-Thomas Co.	Garden Peas O. K.	9 Garden Size		4-14-21 5 years 11-2-21
	FREEHOLD				
266	Monmouth County Farmers' Exchange Earp-Thomas Co.	Red Clover O. K.	792 3 acres	264	2-4-22 5 years 5-20
267	Monmouth County Farmers' Exchange Earp-Thomas Co.	Alsike O. K. Clover	1,490 3 acres	496	2-4-22 5 years 5-9-22
268	Monmouth County Farmers' Exchange Earp-Thomas Co.	Alfalfa O. K. Swt. Clover O. K.	23 1 acre	23	2-4-22 5 years 5-9-22
	ORANGE				
269	Western Soil Bacteria Agency Western Soil Bacteria Agency	Garden Bean O. K.	77 1 acre	77	Not given G. until 7-18-22 5-20-22
270	Western Soil Bacteria Agency Western Soil Bacteria Agency	Alfalfa O. K.	638 1 acre	638	Not given G. until 7-18-22 5-20
271	Western Soil Bacteria Agency Western Soil Bacteria Agency	Red Clover O. K.	1,050 1 acre	1,050	Not given G. until 7-18-22 5-9-22
272	Western Soil Bacteria Agency Western Soil Bacteria Agency	Cow Peas O. K.	7,590 1 acre	7,590	Not given G. until 7-18-22 5-20-22
	SALEM				
253	Dixon & Fogg Mulford Co.	Alfalfa O. K.	340 1 acre	340	8-18-22 1 year 3-17-22
	CAMDEN				
238	Taylor Bros. Mulford Co.	Vetch O. K.			7-21-22 1 year 9-14-22
	SWEDESBORO				
239	H. K. Shoemaker Mulford Co.	Alfalfa O. K.	8,000 1 acre	8,000	8-30-22 1 year 9-19-21

RESULTS OF OFFICIAL ANALYSES—TABLE 61—Continued

Test Number	Town Retail Vendor Wholesale Vendor Commercial Name	Crops and Inoculation		Organisms package per (Millions)	Organisms per acre (Millions)	Date of preparation Guarantee Date of test
	MORRISTOWN					
251	Geo. Loeser Western Soil Bacteria Co.	Vetch	O. K.	78,675 1 acre	78,675	Not given G. until 2-7-22 11-14-21
252	Geo. Loeser Western Soil Bacteria Co.	Lupine	O. K.	12,150 1 acre	12,150	Not given G. until 2-7-22 11-22-21
250	Geo. Loeser Western Soil Bacteria Co.	Alfalfa	O. K.	16 683 1 acre	16,683	Not given G. until 2-7-22 11-3-21
	NEWARK					
244	Muzzy Bros. Earp-Thomas Co.	Alfalfa	O. K.	74 Garden Size	74	4-10-22 5 years 10-19-21
247	Alex. Forbes & Co. Mulford Co.	Garden Peas	O. K.	4 Garden Size		5-25-21 1 year 11-2-4
248	Alex. Forbes & Co. Mulford Co.	White Clover	O. K.	65 1 acre	65	5-2-21 1 year 11-2-21
249	Alex. Forbes & Co. Earp-Thomas Co.	Clovers Red White Crimson Alsike Mammoth Berseem Sapling	O. K. O. K. O. K. O. K. O. K. O. K. O. K.	41 1 acre	41	2-24-20 5 years 11-2-21
254	Alex. Forbes & Co. Mulford Co.	Soy Beans	O. K.	1,092 ¼ acre	4,368	5-2-21 1 year 3-17-22
255	Alex. Forbes & Co. Earp-Thomas Co.	Cow Peas Garden Peas Garden Beans Soy Beans Lima Beans	None O. K. O. K. Poor Fair	22 15 lbs. seed	89	Not given Not given 3-17-22
256	Alex. Forbes & Co. Mulford Culture	Garden Beans	O. K.	37,800 ¼ acre	151,200	12-24-21 1 year 3-17-22
257	Alex. Forbes & Co. Mulford Culture	Red Clover	Fair	42,640 ¼ acre	170,560	12-9-21 1 year 3-17-22
258	Alex. Forbes & Co. Mulford Culture	Lima Beans	O. K.	27 ¼ acre	108	12-24-21 1 year 3-17-22
259	Alex. Forbes & Co. Mulford Culture	White Clover	O. K.	13	52	12-9-21 1 year 3-17-21
260	Alex. Forbes & Co. Mulford Culture	Garden Peas	O. K.	793 ¼ acre	3,172	12-24-21 1 year 3-17-22
261	Alex. Forbes & Co. Earp-Thomas Co.	Clovers Red White Crimson Alsike Berseem Sapling Mammoth	O. K. O. K. O. K. O. K. O. K. O. K. O. K.	43 1 acre	43	Not given Not given 3-17-22
262	Alex. Forbes & Co. Earp-Thomas Co.	Clovers Red White Crimson Alsike Berseem Sapling Mammoth	O. K. O. K. O. K. O. K. O. K. O. K. O. K.	18 5 lbs. seed	72	Not given Not given 3-20-22

RESULTS OF OFFICIAL ANALYSES—TABLE 61—Continued

Test Number	Town Retail Vendor Wholesale Vendor Commercial Name	Crops and Inoculation		Organisms per package (Millions)	Organisms per acre (Millions)	Date of preparation Guarantee Date of test
	NEWARK					
263	Alex. Forbes & Co.	Alfalfa	O. K.	18	71	Not given
	Earp-Thomas Co.	Swt. Clover	O. K.	6 lbs. seed		Not given 3-20-22
	PATERSON					
264	Hart & Co.	Lima Beans	O. K.	10	10	12-30-21
	Earp-Thomas Co.	Garden Bean	O. K.	1 acre		5 years
		Garden Pea	O. K.			3-30-22
		Cow Pea	None			
		Soy Bean	None			
265	Hart & Co.	Alfalfa	O. K.	6	6	12-30-21
	Earp-Thomas Co.	Swt. Clover	O. K.	20 lb. seed		5 years 3-17-22
		Clovers				
245	Hart & Co.	Red	O. K.	2,340	2,340	3-2-21
	Earp-Thomas Co.	White	O. K.	1 acre		5 years
		Crimson	O. K.			10-19-21
		Alsike	O. K.			
		Berseem	O. K.			
		Mammoth	O. K.			
		Sapling	O. K.			
246	Hart & Co.	Garden Peas	O. K.	1,180		10-19-21
	Earp-Thomas Co.	Soy Beans	None	Garden		5 years
		Garden Beans	O. K.	Size		10-19-21
		Lima Beans	O. K.			
		Cow Peas	None			

TABLE 62

Test Numbers of Cultures to Be Noted for Violations, etc.

Company	Unguaranteed	Poor Inoculation	Number of Samples Tested
	261	255	
	262	264	
Earp-Thomas Co.	241	246	20
	255		
	263		
Stulicof Co.		280	15

TABLE 63

Groups of Plants Inoculated by the Same Specific Organism

1. Red Clover	2. Alfalfa	4. Garden Bean	Acacia
Alsike Clover	Bur Clover	Field Bean	Lima Bean
Crimson Clover	Sweet Clover (Yellow)	Scarlet Runner Bean	6. Vetch
White Clover	Sweet Clover (White)	5. Cow Pea	Garden Pea
Berseem Clover	Fenugreek	Partridge	Canada Field Pea
Mammoth Clover		Velvet Bean	Sweet Pea
Sapling Clover	3. Soja Bean (Soy Bean)	Peanut	Brood Bean
			Lentil

TABLE 64

Number of Quantity of Legume Seeds Commonly Sown per Acre

Crop	Rate of Seeding	Estimated Number of Seeds	Crop	Rate of Seeding	Estimated Number of Seeds
	lbs. per A.			lbs. per A.	
Alfalfa	20	5,100,000	Soy Bean	60	150,000
Sweet Clover	20	4,800,000	Field Bean	80	120,000
Red Clover	12	3,300,000	Cow Pea	70	250,000
Alsike Clover	12	8,400,000	Field Pea	120	265,000
White Clover	10	8,000,000	Peanut	30	36,000
Crimson Clover	12	1,500,000	Velvet Bean	12	18,000
Lespedeza	20	7,400,000	Beggar Weed	10	4,200,000
Spring Vetch	60	540,000			
Winter Vetch	60	960,000			

TABLE 65

Summary of Inoculants Tested

All Crops	Alphano Humus Co.	Earp-Thomas Co.	H. K. Mulford Co.	Western Soil Bacteria Co.	Total	All Crops	Alphano Humus Co.	Earp-Thomas Co.	H. K. Mulford Co.	Western Soil Bacteria Co.	Total
All Crops	2	....	....	....	2	Garden Peas	....	1	2	....	3
Peas, Beans, Sweet Peas	....	3	....	....	3	White Clover	....	....	2	....	2
Clovers: Red, White, Crimson, Alsike, Mammoth, Berseem, Sapling	....	4	....	....	4	Lupine	....	....	....	1	1
Soy Beans	....	....	3	....	3	Garden Beans	....	....	2	1	3
Cow Peas	....	....	....	1	1	Red Clover	....	3	1	1	5
Vetches	....	2	1	1	4	Lima Beans	....	....	1	....	1
Alfalfa	....	1	3	2	6	Alfalfa and Sweet Clover	....	5	....	....	5
						Alsike Clover	....	1	....	....	1
						Total	2	20	15	7	44

TABLE 66

Number of Dealers Registered per County

Atlantic	0	Middlesex	1
Bergen	1	Monmouth	2
Burlington	3	Morris	0
Camden	1	Ocean	1
Cape May	0	Passaic	2
Cumberland	7	Salem	3
Essex	2	Somerset	1
Gloucester	3	Sussex	0
Hunterdon	1	Union	0
Mercer	2	Warren	0

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Circular No. 703

May 1944 • Washington, D. C.

UNITED STATES DEPARTMENT OF AGRICULTURE

Method of Testing Legume Bacteria Cultures and Results of Tests of Commercial Inoculants in 1943

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NODULE BACTERIA form an association with the roots of leguminous plants through which nitrogen gas is taken from the air and made available for plant growth. These bacteria are widely distributed in soils, but as there are a number of species, each functioning satisfactorily on certain legumes only, and as strains of the same species vary in their ability to fix nitrogen, the proper nodule bacteria may be lacking in some field soils. They may be isolated from their nodules, tested for efficiency, and propagated to almost any degree under artificial conditions. Commercial laboratories prepare such materials for sale to planters. Whether it is advisable to use artificially prepared cultures of legume bacteria is a matter for local experience to determine.

COMMERCIAL INOCULANTS

At present 11 concerns are engaged in the production of nodule bacteria cultures, or legume inoculants, as they are sometimes called (table 1). For commercial distribution inoculating materials are prepared in "carriers," which are mainly agar (jelly) and moist peat powder. The use of the former within recent years has been declining.

Agar cultures are made by spraying under sterile conditions a suspension of pure nodule bacteria on the surface of hardened sterile special nutrient agar in glass bottles. Usually these bottles are capped with sterilized metal screw caps. The inoculated bottles are incubated at the proper temperature for the organisms they contain, and when good growth is obtained they are packaged and shipped to the merchandiser or consumer. The agar culture must be a pure culture of nodule bacteria, since the growth of contaminants under the extreme artificial conditions may injure the organisms and by appearance may affect the salability of the product.

DEFTS. EX. 21 IDEN.

Defendants Exhibit # 21

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TABLE 1.—*List of commercial seed and soil inoculants sold in the United States in the fiscal year 1943* [1]

Trade name	Carrier	Manufacturer and distributor	Address
Cal-Rhiz Legume Bacteria	Paste (?)	Nelson Laboratories	Stockton, Calif.
Farmogerm	Agar	Earp-Thomas Laboratories, Inc.	Bloomfield, N. J.
Humogerm	Peat	do	Do.
G. L. F. Legume Inoculant	Charcoal	Cooperative G. L. F. Mills	Ithaca, N. Y.
Hansen's Humus Inoculator	Peat	Hansen Inoculator Co	Urbana, Ill.
Certigerm	do	Eastern States Milling Corp.	Buffalo, N. Y.
Kelly's Humus Inoculator	Peat	Kelly Seed & Hardware Co.	Peoria, Ill.
Kelly's Inoculator	Agar	do	Do.
National Cooperative Humus Inoculator.	Peat	Indiana Farm Bur. Coop. Assn.	Indianapolis, Ind.
Legume-Aid	do	Agricultural Laboratories, Inc.	Columbus, Ohio.
Blue Seal Grow Crop Inoculant.	do	Illinois Farm Bur. Purchasing Agency.	Chicago, Ill.
Sooco Legume Inoculant	do	Smith Agricultural Chemical Co.	Columbus, Ohio.
Legumogen	do	The Legumogen Laboratories, Inc.	Delphi, Ind.
Nitragin	do	The Nitragin Co.	Milwaukee, Wis.
Ned-O-Gen Protected Inoculator.	do	The Albert Dickinson Co.	Chicago, Ill.
Badger Brand Inoculation	do	L. Towles Seed Co., Inc.	Milwaukee, Wis.
Cofer's Hitest Inoculator	do	Cofer Seed Co.	Athens, Ga.
Gold Medal Inoculator	do	Louisville Seed Co.	Louisville, Ky.
Hoffman's Inoculator	do	A. H. Hoffman, Inc.	Landisville, Pa.
Kellogg's Rainbo Inoculator	do	Kellogg Seed Co.	Milwaukee, Wis.
M-Inoculator	do	Ed. F. Mangelsdorf & Bro., Inc.	St. Louis, Mo.
May-Bell Inoculator	do	Rose Seed Co.	Louisville, Ky.
Ohio Farmers' Seed Inoculation.	do	Ohio Farmers' Grain & Supply Co.	Fostoria, Ohio.
Red Bird Inoculator	do	Lewis Seed Co.	Louisville, Ky.
Sioux Brand Inoculator	do	Sioux City Seed Co.	Sioux City, Iowa.
Trinity Brand Inoculator	do	Belt Seed Co.	Baltimore, Md.
Wood's Seed Inoculation	do	T. W. Wood & Sons	Richmond, Va.
Superyield Culture	Agar	Strasburger & Siegel	Baltimore, Md.
Uni-Culture	Peat	Kalo Inoculant Co.	Quincy, Ill.
American Tubercle Germ	do	American Field Seed Co.	Chicago, Ill.
Berry's Superior Nodule Germ.	do	Berry Seed Co.	Clarinda, Iowa.
Blue Ribbon Inoculation	do	Allied Seed Co.	Fort Wayne, Ind.
Cornell Keystone Inoculation	do	Cornell Seed Co.	St. Louis, Mo.
Farm Master Nitrogen-Fixing Inoculant.	do	Sears, Roebuck & Co.	Chicago, Ill.
Kalo Inoculation	do	Kalo Inoculant Co.	Quincy, Ill.
Perfection Brand Legume Inoculant.	do	Geo. P. Bemser & Son	Des Moines, Iowa.
Southern States Quality Inoculation.	do	Southern States Cooperative	Richmond, Va.
Seedaid Inoculant	do	Sunfield Seed Co.	Chicago, Ill.
Urbana Culture	Agar	The Urbana Laboratories	Urbana, Ill.
Urbana Humus Inoculator	Peat	do	Do.

[1] Indented trade names indicate material originating in same laboratory as that of the unindented name above; indented names of firms following those of manufacturers denote distributors of subbrand inoculants.

Peat-powder inoculants, on the other hand, are usually prepared commercially by developing a heavy suspension of pure nodule bacteria in aerated liquid nutrients in protected tanks. When at the right stage of growth, the suspension is withdrawn and mixed thoroughly with neutralized peat powder. Further processing may include (1) curing (allowing to stand in a bin or tub for a few days), (2) additional mixing to obtain particle uniformity, and (3) packaging. It is then ready for the retail trade. Prepared in this manner inoculants are not pure cultures, but the presence of other organisms does not seem to affect their efficiency, and they are not sufficiently in evidence as a rule to affect the salability of the product.

The most important ingredient in an inoculant is its content of efficient living organisms. Under the conditions of transportation and storage these organisms may be gradually killed. The useful

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period, therefore, is necessarily limited. This is usually less than a year, and the end of such period is ordinarily shown on the label of the package.

REGULATION OF SALE

For the protection of the planter against products of incompetents and from depreciated material some regulation of the industry is desirable. In addition to certain regulations by the United States Department of Agriculture, the following State institutions are charged with the regulation of the sale of inoculants within their State borders: Indiana Agricultural Experiment Station, at La Fayette; Kansas State Board of Agriculture, at Topeka; University of Maryland, at College Park; New Jersey Agricultural Experiment Station, at New Brunswick; Feed and Fertilizer Control Office, State College, N. Mex.; New York Department of Agriculture and Markets, at Albany; Ohio Department of Agriculture, at Columbus; and Wisconsin Department of Agriculture and Markets, at Madison. Some of these do not make tests of samples.

Where testing of samples is done, it is customary to collect each season sufficient material from each producer to give a satisfactory test of the product. Inoculants are sold by seed and hardware stores, grain elevators, mills, and farm agencies.

METHOD OF TESTING

The function of cultures of nodule bacteria is to produce stimulatory effects by furnishing nitrogen to the legumes to which they are applied. Under practical conditions commercial inoculants are used in the field, and it would seem advisable to test them under similar conditions. Field testing, however, is unsatisfactory on account of several factors, among which may be mentioned the almost universal presence of some species of nodule bacteria and varying climatic and soil conditions, some of which cannot be controlled. In view of these circumstances the greenhouse offers the most prompt, effective, and economical means of testing legume bacteria of the various groups under fairly well-controlled conditions of moisture, light, and temperature.

In greenhouse work of this kind various types of containers and apparatus for growing and watering plants have been employed. Sand is the material usually used to support root growth. A self-irrigating assembly recently has been devised for use in testing numerous samples in limited greenhouse space.[1] Briefly, as modified since its description was published, it consists of a 32-ounce screw-cap-finish bottle from which the bottom has been cut, as a container for a mixture of sand, dolomitic limestone, superphosphate, and potassium sulfate. The mouth end of the bottle is fitted with a plastic cap that has a hole in the center small enough to retain the sand. This end rests on the shoulders of a wide-mouthed pint cream

[1] LEONARD, L. T. A SIMPLE ASSEMBLY FOR USE IN TESTING CULTURES OF RHIZOBIA. Jour. Bact. 45: 523-525, illus. 1943.

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jar holding an acid nutrient solution containing zinc, copper, manganese, iron, and boron salts in very minute quantities. As prepared, the neck of the bottle with cap extends nearly to the bottom of the jar.

After the sand has been completely irrigated, holes are punched in its surface to receive seedlings. The cut end of the bottle is then covered with a Petri dish lid and the whole assembly subjected to steam sterilization in an autoclave. The sand is kept moist by the nutrient solution, which is drawn up by capillarity. The solution is sufficiently acid (pH 2.0) to preclude the survival of any common rhizobia that might drop into the jar when the solution is replenished. Upon reaching the sand the acid solution is neutralized and when carried farther up it becomes available as a nutrient for plants.

Preparation of Subsamples

Before testing moist peat materials, samples are weighed to obtain the net content of carrier in the package. The number of pounds the sample will treat is multiplied by 16, and the net weight of the sample in grams is divided by the number obtained to give the quantity of material in grams necessary for 1 ounce of seed. This is usually very small. Instead of subjecting the inoculant to the possibility of contamination in the process of direct weighing, an approximation of the quantity may be obtained by the use of disinfected, calibrated, narrow glass cylinders, from which the peat powder may be expelled by a disinfected piston of slightly smaller diameter than that of the cylinder. Cylinders and pistons are disinfected by 150° C. dry heat for 1 hour and are allowed to cool before using.

A flame is passed over the lid end before opening samples in cans with removable lids. Sealed cartons and crimped-end cans without a removable lid are opened by cutting with a disinfected knife, into the middle of the side of the container, 3 sides of a rectangular hole, the fourth side acting as a hinge. The carrier is poured from this opening into a properly labeled sterile Petri dish. Labeled sterile vials for subsamples from each package are arranged in holes on a wooden board. A glass cylinder with a piston set at the proper calibration point is tamped in the peat powder until it is filled to the end of the piston. Then, with the aid of an assistant, the subsample is discharged into a sterile vial. Usually five subsamples are taken, but occasionally more are desirable, especially from multiple-group cultures. Lids are replaced or holes are closed with masking tape as soon as possible after part of the contents is removed from the cans.

Agar cultures are not subsampled in this manner. They are prepared according to directions at the time of planting, and subsampling is done by means of sterile pipettes. The common method of preparing agar cultures for use is to add water and shake thoroughly to obtain a homogenized suspension of the nodule bacteria in the bottle.

Seed and its Treatment

Seed of the highest quality obtainable should be used in this work. It should be of uniform size, high in germinating ability, scarified when necessary, and free of debris and of defective, deformed, and broken seeds.

Since commercial legume bacteria cultures are customarily prepared for groups of legumes, each named on the package label, it is

necessary to have on hand a small stock of the various kinds of legume seeds. It is not always possible to test samples on all the species or varieties of legumes for which they are intended, but it is desirable to try them on some of the more common legumes mentioned. Legume seeds ordinarily used in testing cultures are listed below.

Group designation of bacterial sample:	*Plants upon which tests are made*
Alfalfa	Alfalfa and white sweetclover.
Alyce clover	Alyce clover.
Beans	Navy and green beans (Stringless Green Pod and dwarf varieties).
Clover	Crimson, red, and white clovers.
Cowpeas	Cowpea, crotalaria, kudzu, lima bean, and peanut.
Lespedeza	Annual (Kobe, Korean) and perennial species.
Lupine	Blue lupine and other species.
Soybeans	Biloxi, Laredo, Mammoth Yellow, Tokyo, Virginia, and Wilson varieties.
Vetch	Hairy vetch, garden pea (Alaska, Nott Excelsior), and Austrian Winter pea.

Seeds are first washed in sterilized weak peat water to remove clinging dirt particles, then agitated for 5 minutes in a 0.2 percent solution of mercuric chloride. After the disinfecting solution is thoroughly drained away, sterile peat water is used to remove soluble traces of the mercury salt, following which the mouth of the seed container (usually a 16-ounce, screw-capped, wide-mouthed bottle) is covered with a small blanket of sterile absorbent cotton and inverted so as to throw the seeds on the cotton and thereby dry them by draining.

As soon as the disinfected seeds pour easily they are ready to use. Petri dishes containing 0.6 percent sterile agar are prepared, and when hardened the seeds are sown on the surface for germination. Sometimes with large seeds it is desirable to compensate for excessive imbibition by adding sterile peat water. A good temperature for germinating most seed is 70° F.

The number of seedlings that can be planted in an assembly is determined by the growth habits of the resulting plant. Alfalfa, white sweetclover, red clover, crimson clover, and lespedeza may be planted six in a jar; cowpeas, soybeans, crotalaria, mung beans, and lima beans, three; and peanuts, one per jar. If it is desirable to conserve space, two of each of three species of the smaller seedlings may be planted in a jar, or, in the case of soybeans, two of each of two varieties.

Inoculation and Planting

Sterilized planting assemblies, germinated seeds, and subsamples of inoculating material are necessary at the start of the process of inoculation. Sterile Petri dishes and means of disinfecting the instruments (boiling water) should be at hand. A predetermined quantity of sterile water is added to each subsample, usually 20, in a series, and the vials shaken vigorously. Seedlings are placed in sterile Petri dishes in sufficient number to prepare at least 3 replicates of all treatments and 9 of no treatments. The series may also include 9 controls

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inoculated with a known pure culture. The suspended samples are added to the seedlings and the Petri dish agitated to make good contact between seedlings and inoculum.

This method of treatment does not exactly conform to the directions given. The seedlings however, are bathed in a suspension of inoculating material prepared in quantities proportional to those prescribed, and therefore they receive approximately the same quantity as would ungerminated seeds plus those that adhere to the small rootlets.

The seedlings are transferred to previously made holes in the sand by using disinfected forceps. These plantings are usually made in a circle in the center of the bottle, the distance from the side depending on the kind; they are covered by depressing the sand in the middle by means of a ¾-inch rounded metal tamper. A half inch of sterile gravel may then be added to prevent undue evaporation.

Assemblies are labeled on the bottle as to legume, treatment, and date of planting. Replicated treatments consisting of three assemblies are kept together in the greenhouse. Control sets of three are interspersed with the treated sets. Each assembly is placed on a number on the greenhouse bench. Under this number in a notebook the treatments and dates of planting are recorded on a form sheet upon which observations also are placed later. The number of the space used is also placed on the nutrient reservoir to prevent errors when the assemblies are rearranged or removed for observation.

In the greenhouse, control of insects and dust and isolation from ordinary greenhouse operations are necessary. As soon as assemblies are placed, lids may be removed to give better light, aeration, and physical conditions for growth, although the removal presents possibilities for contamination by foreign rhizobia. Since much better growth is obtained in the absence of the lids, however, it is preferable to dispense with them and maintain strict sanitation in the greenhouse.

Plants are usually allowed to grow for about 45 days, unless early maturity or a desire for information requires their earlier removal or it is apparent that a longer period would be advisable.

For some species, as cowpeas, peanuts, or soybeans, in short-day seasons it is desirable to furnish additional light. This is done by using electric bulbs with reflectors, beginning at sundown and continuing 4 or 5 hours into the night. Alfalfa, clovers, and vetch produce satisfactory results from the light coming through the greenhouse glass during the winter season.

In the case of vining or high-growing plants, supports are necessary to prevent falling or intertwining. This is done by means of disinfected hardware-cloth cylinders that will fit snugly around the sand bottles.

Observations and Performance

The main points to consider for effect of inoculating material are the differences between treated and control plants in nitrogen content, dry matter, color, size of plants, and nodule formation. In special cases, nitrogen analysis may be desirable, but for the ordinary estimation of bacterial stimulation color is a good index of nitrogen fixation or lack of it. Color may be observed more satisfactorily while the plants are in the greenhouse.

Observations on color and height are preferably made before the

plants are disturbed by removal from bottles. After roots are freed from sand by washing in water they are examined in a bottom-illuminated glass water bath, originally described by Marsh and Leonard [2] and later modified by Marsh to give side light. By means of this bath roots floating in water are easily measured and estimates made of nodule numbers and their location.

If observations on color show that plants from treated seeds are dark green and the corresponding control plants are light or yellowish green, it is a positive indication that organisms and the plant through symbiosis have produced satisfactory results.

TABLE 2.—*Summary of results of greenhouse tests on samples of legume bacteria cultures of commercial origin in the fiscal year 1943*

[U=unsatisfactory; S=satisfactory]

Trade name	Alfalfa		Clover		Cowpea		Lespedeza		Soybeans		Beans		Vetch		Total		Sources total [1]	
	U	S	U	S	U	S	U	S	U	S	U	S	U	S	U	S	U	S
Cal-Rhiz Legume Bacteria	0	5	0	3	0	2			0	2	0	2	0	10	0	24	0	24
Farmogerm	4	0	2	2									1	3	7	5		
Humogerm	2	0	2	0									0	2	4	2	11	7
G. L. F. Legume Inoculant	2	2	4	0									3	1	9	3	9	3
Hansen's Humus Inoculator	0	2	4	2									0	6	4	10		
Certigerm	0	3	0	3			3	0	1	2			0	3	4	11		
Kelly's Humus Inoculator	0	2													0	2		
Kelly's Inoculator			0	2											0	2		
National Cooperative Humus Inoculator	0	2	0	2			2	0	0	2					2	6	10	31
Legume-Aid	0	2	0	2	0	2	0	2	0	6	1	4	0	11	1	29		
Blue Seal Grow Crop Inoculant	0	7	0	7					0	1					0	15		
Sacco Legume Inoculant	0	2													0	2	1	46
Legumogen	0	4	0	4					0	2					0	10	0	10
Nitragin	0	5	0	6	0	2	0	6	0	4	0	4	2	11	2	39	2	39
Nod-O-Gen Pretested Inoculator	0	6	0	6	0	2			0	5	0	5	0	10	0	25		
Badger Brand Inoculation	0	4	0	2											0	6		
Cofer's Hitest Inoculator	0	2	0	2	0	3	0	3	0	3	0	3	0	2	0	16		
Gold Medal Inoculator	0	2	0	2	0	3	0	3	0	3			0	2	0	15		
Hoffman's Inoculator	0	2	0	2	0	3	0	3	0	3			0	2	0	15		
Kellogg's Rainbo Inoculator	0	2	0	2					0	3			0	2	0	9		
M-Inoculator	0	2	0	3	0	2	0	3	0	2			0	2	0	14		
May-Bell Inoculator	0	4	0	4	0	3	0	3	0	3			0	2	0	19		
Ohio Farmers' Seed Inoculation	0	2	2	0											2	2		
Red Bird Inoculator	0	2	0	2									0	2	0	6		
Sioux Brand Inoculator	0	2	0	2											0	4		
Trinity Brand Inoculator	0	3	0	2			0	6	0	2			0	2	0	15		
Wood's Seed Inoculation	0	2	0	2	0	5	0	3	0	3	0	3	0	5	0	23	2	181
Superyield Culture									0	6	0	4	1	15	1	25	1	25
Uni-Culture	0	4	0	4					0	4					0	12		
American Tubercle Germ	1	3	0	4	0	3	0	3	0	3					1	16		
Berry's Superior Nodule Germ	0	2	0	4	0	3	0	3	0	1			0	3	0	18		
Blue Ribbon Inoculation	3	0							0	6					3	6		
Cornell Keystone Inoculation	0	4	0	4	0	6	0	3	0	3			0	2	0	22		
Farm Master Nitrogen-Fixing Inoculant	0	2	2	0					0	1					2	3		
Kalo Inoculation									0	1			0	4	0	5		
Perfection Brand Legume Inoculant	0	3	0	3			3	0	2	4					5	10		
Southern States Quality Inoculation	0	4	0	4											0	8		
Sunfield Inoculant	0	4	0	4											0	8	11	106
Urbana Culture	0	2	0	2	5	0	0	6	0	6	0	6	0	2	5	24		
Urbana Humus Inoculator	0	2	0	4									0	2	0	8	5	32
Total	12	101	16	97	5	41	8	47	3	81	1	31	7	106	52	504		

[1] This column gives test results with products of individual manufacturers without particular reference to trade names.

[2] MARSH, F. W., and LEONARD, L. T. AN APPARATUS FOR THE SUPERFICIAL EXAMINATION OF ROOTS AND NODULES. Soil Sci. 26: 403-404, illus. 1928.

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If upon root examination nodules are found to be uniformly present on the dark-green plants from treated seeds and none or only a few on the control-plant roots, there is no doubt as to the effectiveness of the culture.

If plants are yellowish or not a normal green and no nodules are formed, it is evident that the treatment did not contain the proper organisms and is therefore unsatisfactory.

If foliage is yellow or not a normal green and nodules are observed uniformly on the roots, it shows that the culture is not adapted to the host and consequently is unsatisfactory. This condition, however, is found only occasionally.

TESTS OF COMMERCIAL INOCULANTS IN 1943

Using the method here described, the Bureau of Plant Industry, Soils, and Agricultural Engineering during the year ended June 30, 1943, tested numerous samples of inoculating materials seasonally collected in the open market from various parts of the country. This work was done in accordance with an act of Congress making appropriations for soil and fertilizer investigations "* * * including the testing of cultures procured in the open market for inoculating legumes, other crops, or soil * * * *." The act provides also for publication of the results of the tests together with the names of the manufacturers "* * * if any such samples are found to be impure, nonviable, or misbranded * * *." Results of the 1943 tests are thus summarized in table 2, reference to table 1 showing the names of producers. The manufacturers of samples found to be unsatisfactory were notified of the results, so that steps could be taken to improve the product for the 1944 season.

U. S. GOVERNMENT PRINTING OFFICE: 1944

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DEFTS. EX. 22 IDEN.

NITROGEN from the AIR

CONVERTED INTO

FOOD for PLANTS

BY

FERGUSON'S NITROGEN BACTERIA

"Living Nitrate Factories for Field and Garden."

A COMPOSITE CULTURE

One bottle available for all legumes.

Four ounces properly applied to the seed for an re of ground will put as much nitrates into the il as can be obtained in **two tons** of high grade mmercial fertilizer, with less labor and less pense.

THIS MEANS

Profitable crops on poor or sandy soils
Restoration of worn out lands
Increased crops on good lands

HOMEWOOD NITROGEN CO.

Laboratories, 117 West Main Street

Richmond, Virginia

usiness Office, 51 Liberty Street, New York City

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1913, Second Edition } by Homewood Nitrogen Co

HISTORY

For centuries the farmers of the world have known that crops have been larger when grown on a field that had previously been planted to clover or other legumes. Why this was so was not understood, but a German scientist and chemist, Professor Heilriegel, of the University of Frankfort, about the year 1860, discovered that the nodules on the roots of these legumes contained active colonies of bacteria. After long experiments he was able to isolate these bacteria. The scientific fact of the utility of the bacteria in increasing the growth of the legumes was thus observed and established, but no efficient method was discovered for their commercial utilization. These researches were continued by the scientists of Germany and many years later were taken up by the experts of the Agricultural Department at Washington and also by the experts of some of the agricultural stations of the various states. Prominent among these was Dr. Meade Ferguson, State Bacteriologist of the Commonwealth of Virginia. For about ten years Dr. Ferguson continued his tests and experiments, putting up thousands of packages in different forms, sending them to the farmers and official stations for practical and field tests throughout a large territory. In this way a large amount of data of the greatest value was obtained, showing the increase of the crop, the enrichment of the soil, and the best methods and means of obtaining the same. So successful were these tests and demonstrations that today Dr. Ferguson has justly earned the position as one of the leading scientists and bacteriologists of the world. The results of these discoveries have been made available to the public through the products of the laboratories of the Homewood Nitrogen Company at Richmond, Virginia.

WHAT ARE NITROGEN-FIXING BACTERIA?

Nitrogen Fixing Bacteria are small living organisms having the power to take nitrogen from the air, convert it and fix it in a form available for plant life. In this way they supply to the soil the nitrates which are the most expensive and valuable of all fertilizers. The bacteria not only feed the plant, acting as their host, but also store up surplus supplies of nitrogen in the form of nodules upon the roots of such host plant.

These bacteria thrive on certain leguminous plants, of which there are thirty-seven varieties. The principal of these are alfalfa, clovers, beans, peas, vetches, lupines, soy beans, etc. The bacteria are not recommended as being directly applicable to the cereals, such as wheat, oats, corn, barley, etc., although such crops can be directly benefited by planting with them some inoculated legume seed such as field peas, the leguminous plant in such case furnishing the nitrates for the cereals as well as for itself. They also benefit the cereals indirectly when such crops are planted on soils that have previously grown inoculated legumes.

DR. FERGUSON'S COMPOSITE CULTURE

Dr. Meade Ferguson, State Bacteriologist of the Commonwealth of Virginia, has discovered a method by which he has achieved most remarkable and valuable results in the production of a composite culture which is available for any leguminous plants. Through his work it is now no longer necessary to have a separate inoculation for alfalfa, a separate inoculation for clover, a separate bacteria for vetch, a separate bacteria for beans, a separate bacteria for peas, etc., throughout the entire list. Dr. Ferguson's composite culture can be used with equal effectiveness upon any or all of the legumes.

NITROGEN AND ITS DEPOSIT

Nitrogen is the most important and most expe
of the essential constituents for a good agricu
soil. It is rapidly depleted by the growing and
moval of grains and many other crops. This re
in the impoverishment of the soil, the diminution
the crops, and the steady depreciation of farms. T
process has been exemplified in the steady exhau
of the soil of the abandoned farms of New Engla
A bankrupted soil has meant deserted comm
This process is now being repeated in the great
ing region of our middle, southern and western st
Nitrogen, however, in the form of nitrates can be
back in the soil from the atmosphere itself through
presence and activity of these nitrogen fixing bacte
Every acre of ground is covered with atmosphere c
taining about 77.77% by weight of pure nitrogen
the equivalent of about 1,680 pounds of nitrogen
the square foot, or nearly 40,000 tons of nitrogen
the acre,—a simply inexhaustible supply when
available for plant life through the activity of
bacteria. Practically all soils, even those that h
been most exhausted, can be rehabilitated in this w
If land has turned sour it must first be limed so as
neutralize its acid condition before the bacteria c
do their work.

OTHER FERTILIZERS—COST AND INEFFICIENCY

The means generally adopted to restore nitro
to depleted soils are:

(a) The application of barnyard manure, s
other organic matter about the farm. As this ma
terial contains only about one-third of one per ce
of nitrogen, the supply at hand is hardly ever ade
to return to the soil the amount of nitrogen, remo

cropping. Furthermore, such fertilizers often con- other elements that are a positive detriment to farmer and involve much additional work.

(b) Commercial fertilizers are manufactured and but their supply is limited and their cost is high. igh-grade commercial fertilizer, containing only 4% nitrates, sells upon the market for over $40 per . A ton of such fertilizer puts into the soil only out 80 pounds of nitrates against a total enrichment ctimes found of 850 pounds of nitrates, deposited one acre of ground through the use of one four- ce bottle of Ferguson's Nitrogen Fixing Bacteria, n properly applied through inoculated seed.

(c) The contemplated process of making nitrates the electric current does not offer much relief, be- se the price of nitrates even when manufactured ll not be much if any lower. This makes it un- ractive to the practical farmer.

COMPARATIVE EFFICIENCY OF FERGUSON'S COMPOSITE BACTERIA WITH OTHER BACTERIA

Ferguson's Composite Nitrogen Fixing Bacteria is de according to a new process of culture. In order show the comparative value of Ferguson's with ers, tests were made by E. B. Fred, Bacteriologist the Virginia Experimental Station, under the ited States Department of Agriculture.

In order to test the value of different inoculating ro-cultures now employed extensively for artificial culation, samples were secured and bacterial counts de. All nitro-cultures were diluted as directions ed for, and one pint samples were set aside from ch the counts were made. The following table es the number of bacteria per c.c. in the four cul- es examined:

	Ferguson's Composite Bacteria	Old Process	Agr. Dep't Washington	Farmogerm
Red Clover	180,000	51,600	3,400	50
Alfalfa	205,500	41,000	5,000	38
Cow Pea	220 000	51,600	7,000	42
Soy Bean	85,000	53,000	2,700	36
Crim. Clover	250,000	53,000	6,000	55

Old Process Cultures were those originally prepared by Dr. Meade Ferguson, formerly of Virginia Experimental Station.

Agricultural Department, Washington, cultures, were prepared by the Agricultural Department of the United States.

Farmogerm were cultures prepared by the Earp-Thomas Co. of New Jersey.

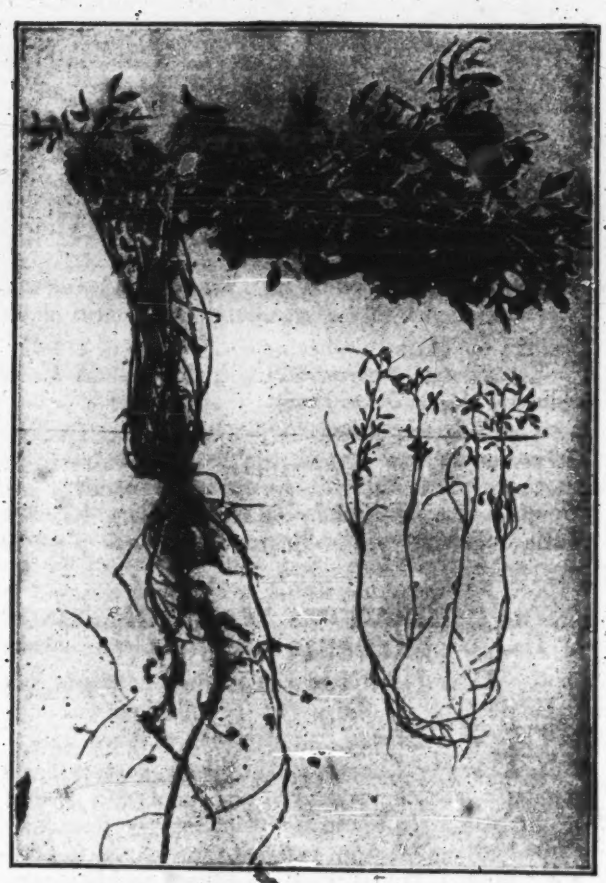
The Ferguson Composite Nitrogen Fixing Bacteria is unique in that it possesses features unknown to any other formula, viz., its *composite nature*. Dr. Ferguson has solved the problem of making Nitrogen Fixing Bacteria applicable to any legume plant in any soil.

WHAT FERGUSON'S NITROGEN-FIXING BACTERIA WILL DO

1. These bacteria furnish the best and most valuable fertilizer in the world, in unlimited quantities and at only a nominal cost of money or labor.

2. They take nitrogen from the air, convert it into nitrates available for plant food, and then give it to the plant. In return the plant supplies them with carbo-hydrates, thereby enabling the bacteria to grow and reproduce themselves at an almost incredible rate.

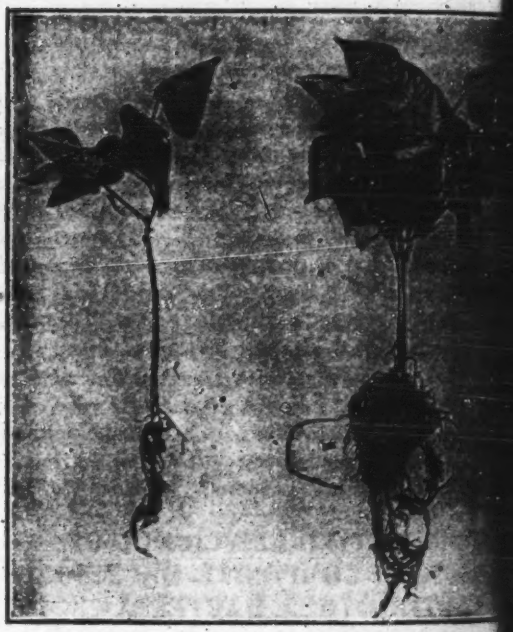
3. **They increase crops,** under ordinary conditions from 25% to 60%, with no extra work. They make possible profitable crops on absolutely barren soil, such as sea sand or even coal ashes. They also improve the crops on good farm land.



ALFALFA PLANTS—Planted at the same time and in the same soil and under the same conditions.

Those on the left were inoculated with Ferguson's Nitrogen-Fixing Bacteria. Those on the right were not inoculated. Notice nodules on the inoculated plant.

(This illustration is an exact reproduction from the official government bulletin, No. 159, of the Virginia Agricultural Experiment Station.)



SNAP BEANS—Grown in coal ashes.

The one on the right was inoculated with Ferguson's [illegible] Fixing Bacteria; that on the left was not inoculated. Notice [illegible] on inoculated plant.

(This illustration is an exact reproduction from the official [illegible] ment bulletin, No. 154, of the Virginia Agricultural [illegible] Station.)

4. They store up on the roots of such [illegible] the excess nitrates which the plant does not use. [illegible] is done in the form of nodules, varying in size [illegible] mere speck to about a half inch in diameter. [illegible] reports show this reserve supply of nitrates that [illegible] in the ground has been in some tested cases [illegible] 850 pounds per acre, or more nitrates than are [illegible] tained in several tons of the average commercial [illegible] tilizer.

. **They gather moisture** from the air at the e time that they gather nitrogen, and also make it ilable for the future use of the plant. This enables oculated field to withstand some droughts or dry ns that would otherwise destroy the crop.

. They work night and day *making money* for the ner.

WHAT THEY WILL NOT DO

. They will not live if exposed to strong sunlight extremes of heat or cold. After they are once ted and take up their work in the ground they live through a cold winter or hot and dry summer out injury.

. They will not do well in a sour soil. If the l is sour it should be well limed according to its ition, with from 500 lbs. to two tons of ground rbonate of lime or its equivalent, per acre, and then inoculated seed will produce large increase. e our booklet on "Sour Soils and How to Test m."

. They will not do the plowing or cultivating of ld when the farmer has been unable or too lazy to perly prepare the soil for any crop, but if given the ortunity they will put the field in better condition succeeding crops.

DIRECTIONS FOR USING

t. Do not open the bottle of bacteria before you ready to use it. If you do, it is liable to spoil. en everything is ready, uncork the bottle and place the sugar contained in the small envelope supplied the bacteria, and shake thoroughly until dissolved. sugar supplies a food for the bacteria and makes active until such time as the seed germinates.

ld. Then fill the bottle with water. The water ly acts as a carrier, to spread the bacteria to all eeds. The amount of water required varies somet with the different varieties of seed. The small h, alfalfa and all the clovers, do not readily take up

moisture, and so a small quantity of water is sufficient. Vetch, peas and beans, having much greater bulk, require a larger amount of water. Therefore when inoculating vetch, peas or beans fill the bottle a second time with water and add to the first mixture.

3rd. Then sprinkle by hand this liquid upon the seed, which has been heaped upon a cleanfloor or placed in a clean vessel, until every seed is slightly moist. It is essential that every seed should be slightly moist but not wet. If too much water is used then the seeds can be quickly made dry enough to sow by scattering or mixing a little dry sand or fine sifted coal ashes through the mass.

4th. As soon as the seed is dry, sow at once and harrow in at once. Avoid strong sunlight and do your sowing early in the morning or late in the evening, or on a cloudy or rainy day. Strong *sunlight kills* every kind of bacteria.

CAUTION

Do not uncork the bottle until you are ready to immediately inoculate the seed with it. Exposure to the air is almost certain to contaminate it with other bacteria.

Do not mix this bacteria in unclean or rusted vessels or with hot water or with ice water. *Remember* that it is possible to kill the bacteria by excess heat or excess cold, or with certain acids, such as are found in sour soil.

Do not apply the bacteria to the seed in the *sunlight* or expose to the sunlight to dry after mixing. *Remember* that strong sunlight kills every form of bacteria. The best results are obtained when planting is done in the early morning or late afternoon or on cloudy days.

We do not advise transference of soil, for in a large percentage of cases such transfers bring detrimental bacteria and fungus growths that are injurious to the crop, and especially to the future crops in subsequent years.

LIFE OF BACTERIA

All the bacteria sold by the Homewood Nitrogen Company is guaranteed as viable for a limited period after shipment, the date of such guarantee being stamped on each package. Dr. Ferguson's experience has shown, however, that large quantities of his bacteria have been kept alive and in an active state for two years and over without deteriorating, making Dr. Ferguson's composite nitrogen fixing bacteria in this respect incomparably superior to any other on the market. Official investigations by the Geneva Experimental Station of the State of New York showed that in all the other commercial nitro-cultures which were examined by them, practically all were devoid of living bacteria after the first few weeks. Such cultures would be not only useless but in many instances detrimental.

BACTERIA INCREASE CROPS

Careful tests show that certain leguminous crops are increased from 25% to 60% through the use of nitrogen fixing bacteria. This involves practically no additional labor, as the entire process of preparing the seed for an acre of ground can be done within less than an hour's time and with no additional cost for machinery or equipment. The use of Ferguson's Composite Fixing Bacteria is particularly attractive to the farmer, as it not only shows a great saving in initial cost but a great saving of labor necessary in the spreading of ordinary commercial fertilizers. The simplicity of the process of inoculating the seed will be seen by reading the directions in this booklet.

BACTERIA HARMLESS

Ferguson's Culture of Nitrogen Fixing Bacteria is absolutely harmless. It cannot hurt the hands of any one handling it, nor does it cause disease in any way. It is absolutely safe.

TESTIMONIALS FROM PRACTICAL FARMERS

(Copy of letter of George Carragan, President of the First National Bank of Bayonne, New Jersey)

RESULTS WONDERFUL.—About three years ago I had occasion to use some of Ferguson's Composite Nitrogen Fixing Bacteria on my farm at Somerville, New Jersey. At that time I used it on several acres of alfalfa and on a field of soy beans. I also used on my meadow land where inoculated clover seed was planted with timothy. The results of the inoculation were indeed satisfactory, and wonderful. The alfalfa has grown during the time since the planting, giving four cuttings each year, which considered very remarkable by the farmers about Somerville, Jersey. The crop of hay on the meadow lands was much larger than it had been in previous years.

The farm now not only produces an abundance of hay for the feeding of the stock, but I have sold several tons each year. I have taken great pleasure in recommending Ferguson's method of fertilization and believe that it will be of great value to farmers when properly applied.

October 25, 1912. Very cordially yours,

Geo. Carragan

(Copy of letter from W. R. Comfort, President Realty Co., Director Dairymen's Mfg. Co., Broadway Trust Co., Etc., New York City)

SUCCESS WITH ALFALFA.—For three years I have been trying to secure a stand of alfalfa on some land of poor quality in the edge of the Everglades near Miami, Fla., but have been unable to do so until this year when I used the alfalfa seed inoculated with Ferguson's Nitrogen Fixing Bacteria prepared by your Company. I am very pleased to tell you that this has resulted in a very wonderful growth and satisfactory stand of alfalfa. I am more than pleased at the results and believe that the use of Ferguson's Nitrogen Fixing Bacteria will be of great value to the farmer when properly used.

Very truly yours,

W. R. Comfort

(Copy of letter of Mr. C. H. Griffing, formerly Manager of the Woodland Department of the International Paper Co.)

RESULTS OF LONG SERIES OF TESTS.—For several months experiments have been conducted under my personal supervision of the effect of inoculating seeds with Ferguson's Nitrogen Fixing Bacteria. I have seen seeds of alfalfa

...mson clover, white clover, alsike clover, fenugreek, string ...ans, lima beans, soy beans, field peas, garden peas, sweet ...ea, and other legumes, treated with this bacteria and planted ...different kinds of soil, varying from good rich soil to ...sal ashes and ocean sand. Uninoculated seeds were planted ...separate pots, under identically the same conditions of ...ed, soil and moisture. These tests were conducted with ...reat care to find out just what effect moistening with Fer...son's bacteria would have. The results showed a sub...antial increase where the inoculation was used, and in some ...stances a very remarkable growth.

I also examined a field where a test was made upon a ...ge scale. In California twenty-one acres were planted ...th fenugreek inoculated with Ferguson's bacteria and five ...res without inoculation. The ground where this test was ...ade was unusually rich so that less difference would be ...pected. The growth above ground, however, showed a ...bstantial increase in favor of the inoculated seed, while ...e roots and growth below ground showed at least twice ...e growth and number of nodules compared with that of ...e uninoculated. This increased root and nodule growth ...as undoubtedly responsible for and the cause of the superior ...ndition of the plants above ground. The soil was also ...doubtless permanently enriched by the nitrates deposited in ...e nodules.

From my investigation and experience I am convinced that ...erguson's Nitrogen Fixing Bacteria are of very great prac...ical value to the farmers and land owners when properly ...plied.

Very truly yours,

C. H. GRIFFING.

ST WITH COTTON.—In the Spring of 1910, at the request ...f Mr. Hurst, who has a large cotton plantation at Glover, ...Soto County, Mississippi, I went from New York to his ...antation to make and superintend a test of Ferguson's ...itrogen Fixing Bacteria in connection with the growth of ...tton plants. The test was made on his plantation of about ...ree hundred acres then in cotton plants, selecting there...om two acres of average conditions for the purpose. The ...il was a dark, rich loam, Mississippi bottom land. On it ...d been grown cotton for many years, practically without ...terruption. In recent years a large amount of fertilizer ...d been used, the cost of the fertilizer being reported at ...er $30 per acre per year. With this fertilization the land ...d been producing about two bales of cotton to the acre.

At the time of starting the test, in April, the cotton ...nts on the plantation were about six inches in height.

On the two acres selected Canadian field peas were drilled into the ground about eight inches distant from the cotton plants. These field peas had first been inoculated with Ferguson's Nitrogen Fixing Bacteria, the process of inoculating taking only a few minutes. The field peas made a rapid and strong growth, benefitting the cotton plants, so that they showed a noticeably increased growth over the surrounding cotton plants in a short time. The results obtained on the two acres, where the inoculated peas had been planted, showed a marked increase in the crop, making a gain of about half a bale of cotton to each acre or the equivalent of a twenty-five per cent increase over the crop on adjacent acres.

In obtaining there results the cost of the use of Ferguson's Nitrogen Fixing Bacteria and of the field peas (of which about 20 pounds were used to the acre) was only a small fraction of the cost of other fertilizers and the labor in drilling the field peas was much less than spreading the fertilizer for the same ground.

I believe that a substantial increase in the cotton crop in many sections of the country can be economically and profitably secured through the proper use of Ferguson's Nitrogen Fixing Bacteria.

FRANK M. HEMPHILL, Newark.

(LETTER OF J. M. COBB, ATAHSON, N. C.)

INCREASE FIVE FOLD.—Your favor received asking how I liked your nitrogen fixing bacteria. I must say to give my honest opinion that it would increase our average yield five-fold if it is properly used. It gives promise of doing more for me. Your directions were closely followed and experiment plot made on the road showing travellers the benefit to be derived from it.

J. M. COBB.

(LETTER OF L. A. LEWIS, OF THE ALBRO J. NEWTON CO., BROOKLYN)

RESULTS OF A POT TEST.—I got a test bottle of Ferguson's nitrofying bacteria some time ago as I wished to make a test on alfalfa seed that I had bought for this year's sowing. I got two plant jars of the same capacity, filled the two with the same (rich dirt) and sowed the seed. The jar in which I used the bacteria came up stronger, showed a better color, grew faster and with a ranker growth, which has convinced me it is all you claim for it.

Yours very truly,

L. A. LEWIS.

ENDORSED BY
NEW YORK CITY FARM SUPERINTENDENT.

I made a test of Ferguson's Composite Culture of Nitrogen Fixing Bacteria. As Superintendent of the farm owned by the City of New York and conducted by the Department of Charities and Correction of New York City, in connection with the city penitentiary, I inoculated with this bacteria directly beans and peas, and used inoculated humus on lettuce, cucumbers, cabbage, beets and other vegetables. The results showed a marked increase where the inoculated seeds and humus were used as compared with the same kind of vegetables not inoculated. The increase in the crop certainly more than paid for the slight cost of applying the bacteria.

I shall be glad to avail myself of its use again as occasion arises, as I am confident that the system can be of great benefit to anyone who will properly use it.

D. CLARK.

RESULTS AMAZING.—Used inoculating material on clover, timothy and grass in hard clay soil, previous crop potatoes—we wished to make a lawn. The inoculating material left we mixed with sand and threw it over the rest of the place. The result was amazing—we had it cut numerous times, and last week it was in splendid condition.

MRS. C. T. HIPPEY, Roanoke, Va.

U. S. DEPARTMENT OF AGRICULTURE
FARMERS' BULLETIN No. 315

"The peculiar value of legumes for increasing and maintaining the fertility of soils is due to certain bacteria which develop nodules upon the roots of leguminous plants and which have the unique power of rendering the free Nitrogen of the atmosphere available for plant growth. Without these bacteria, legumes, like other crops, exhaust the soil of its combined Nitrogen. In many regions certain types of these important bacteria are abundant in the soil; in other localities they must be imported, either by distributing soil from a field where they are known to be present, or by using pure cultures of the proper organisms grown under artificial conditions. The old method of importing bacteria by distributing soil from fields containing them is not only expensive, but there is a very great danger of spreading weeds and destructive crop diseases, as well as the desirable bacteria. Under modern conditions, therefore, it is wisest to depend chiefly upon the intelligent manipulation of pure cultures for inoculating leguminous crops.

"Recent improvements in the methods of preparing and distributing pure cultures for inoculating leguminous crops have obviated many of the difficulties previously experienced in their use."

PRICES OF FERGUSON'S COMPO
CULTURE NITROGEN-FIXING
BACTERIA

Available for Clovers, Alfalfa, Beans, Vetches, Lupines, Soy Beans, and other Legumes.

Bottle of bacteria sufficient to inoculate seed for one acre . . . . . .

Bottle of bacteria sufficient to inoculate seed for five acres . . . . . .

Small bottle of bacteria sufficient to inoculate garden or quarter acre . . .

The one acre package, also garden size can be sent through the mail. The five acre will be sent by express prepaid.

Orders will be filled by mail if sent direct Company, upon personal check, cash or mone enclosed.

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1046 DEFENDANT'S EXHIBIT 23.

THE EFFECTS OF NONBENEFICIAL NODULE BACTERIA ON AUSTRIAN WINTER PEA

BY

LEWIS T. LEONARD AND W. R. DODSON

(Contribution from Bureau of Chemistry and Soils)

Reprinted from Journal of Agricultural Research
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(Pages 649-663)

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U. S. Government Printing Office : 1933

1047 TABLE 8.—NITROGEN FIXATION IN GREENHOUSE PLANTINGS OF AUSTRIAN WINTER PEAS

Inoculation treatment	Duration of experiment	Nodules per plant	Dry weight (50 plants)	Nitrogen	Total Nitrogen
		Average number	Grams	Per cent	Gram
None	Oct. 28, 1929 to Dec. 12, 1929 (45 days)	....	13.00	2.60	0.339
473		....	12.50	3.33	.416
484		....	12.50	2.38	.300
510		....	15.50	3.04	.471
511		....	23.00	2.94	.675
512		....	21.50	3.08	.664
513		....	21.50	2.15	.461
515		....	23.00	2.40	.550
518		....	20.50	3.09	.633
None	Feb. 14, 1931 to Apr. 8, 1931 (53 days)	3	15.60	1.30	.203
510		15	18.50	2.86	.532
511		16	20.75	2.74	.568
513		96	15.90	1.22	.194
518		13	26.85	2.36	.635
None	Apr. 4, 1931 to May 25, 1931 (51 days)	1	12.80	1.72	.220
512		15	13.80	2.70	.372
513		135	13.20	2.04	.269
548		90	11.40	1.66	.189
549		99	12.20	1.68	.205
550		122	11.60	1.66	.192
512+513		29	11.70	1.94	.227
513+$NaNO_3$		44	14.90		

In the first experiment, culture 510 apparently gave poor results, so far as dry weight and total nitrogen are concerned, yet the percentage of nitrogen rather closely parallels that of the other beneficial cultures. Considering matters on the basis of total nitrogen apparently fixed by nodule organisms, it will be noted that the plants from only one culture (484) contained less nitrogen than the control. Apparently the so-called nonbeneficial cultures were slightly beneficial when the quantity of nitrogen fixed is considered. However, in the second experiment the nitrogen fixed by the nonbeneficial culture (513) was actually less than that of the control and much less than that of any of the three beneficial cultures employed and in the last experiment this nonbeneficial culture was slightly better than the control. When the two types of organisms (512 and 513) were used together the total nitrogen fixed was not greater than that fixed by the nonbeneficial organism alone. However the addition of 0.1 per cent sodium nitrate to the solution originally used to moisten the sand (subsequent waterings being made without nitrogen additions) helped the plants inoculated with a nonbeneficial organism considerably.

Source of the Nonbeneficial Organisms

The fact is evident that nodule bacteria beneficial and detrimental to Austrian winter peas exist in some of the soils around Jeanerette, La., the nonbeneficial ones evidently predominating. In seeking an explanation for the presence of these organisms it was found that plants of Louisiana vetch (*Vicia ludoviciana* Nutt.) grew plentifully and luxuriantly around the fields and in waste places. The roots of these wild plants are well populated with nodules. It is therefore reasonable to believe that the organisms causing these nodules are abundantly present in the soil. It is customary to consider that the organisms of the common vetch will cross-inoculate with garden

1047 peas, field peas, Austrian winter peas, and other legumes although data concerning the group relations of Louisiana vetch have not been worked out. However, acting on the supposition that the bacteria of this legume are related to those of the Austrian winter pea but not satisfactorily adapted to the latter, the writers made isola-

tions from nodules for comparison with the nonbeneficial bacteria obtained from Austrian winter peas.

After the cultures from Louisiana vetch were purified they were applied to Austrian winter pea and Louisiana vetch seed in the greenhouse under controlled conditions, as indicated in Table 8. These cultures produced nodules on their specific hosts. The results with Austrian winter peas indicate that these Louisiana vetch nodule organisms produce the same effect as those of the nonbeneficial type whose effects have been previously noted in this paper. From April 4 to May 25, 1931, the Austrian winter-pea plants grown from seed treated with three cultures of nodule organisms of Louisiana vetch had much the same appearance as those from seed treated with a nonbeneficial culture isolated from Austrian winter pea nodules. (Fig. 3.) As compared with the control, Louisiana vetch organisms (548, 549, and 550) contributed no nitrogen to the Austrian winter peas; in fact they caused a slight loss. (Table 8.)

Nodules produced by Louisiana vetch cultures were plentiful, scattered, and globular. Nodules of this type are shown in Figures 4 and 5 in comparison with nodules produced by beneficial and nonbeneficial cultures from Austrian winter peas. Characteristic nodule assemblies are more distinct in greenhouse samples than in field samples. (Fig. 2.) Table 8 shows that the average number of nodules per plant is considerably higher when poor cultures of Austrian winter peas and Louisiana vetch were used than when beneficial organisms were used. The close similarity of response of Austrian winter peas to nodule organisms from Louisiana vetch and from apparently parasitized Austrian winter peas is indicative of the origin, of the unadapted strains present in the soils studied.

Summary

Cultures of nodule bacteria from poor and vigorous Austrian winter-pea (*Pisum arvense*) plants from Jeanerette, La., have been compared with nodule bacteria from closely related legumes in field and greenhouse tests.

Drainage obtained by ridging the soil had but little effect, and lime and basic slag had practically no effect on the crop produced.

A favorable well-defined effect in quantity and quality

of crop was evident in Austrian winter peas treated with efficient nodule bacteria in soil known to contain inefficient nodule-producing organisms.

Evidence has been presented which shows that the nodule organisms of the Louisiana vetch (*Vicia ludoviciana*) reduce the yield of Austrian winter pea on account of their unadaptability to this host.

1048 DEFENDANT'S EXHIBIT 24.

Reprinted from the Journal of the American Society of Agronomy, Vol. 17, No. 10, October 1925

SOME TESTS WITH SOILGRO[1]

LEWIS T. LEONARD[2].

"The quest continues for a mixed culture of soil bacteria which, when added to the soil, will exert an extraordinary stimulating effect on all plant growth. Starting with Alinite about 30 years ago, after the first successful trials in the inoculation of legumes with pure cultures of nodule bacteria, there have been produced preparations named Geonitrine, Nitrobacterine, Humogen, Phosphogerm, All Crop Farmogerm, Bac-su-muh, Biological Fertilizer, Hu-fi-

1049 teria, Nitragine, U-Kultur, Vitamite, Humophos, Nitrobacter Soil Vaccine, Dextrogerm, Verdure, and others for which far-reaching claims have been made. By the use of these preparations nitrogen from the air was said to be fixed in the soil and to be made available for all crops; while the transformation of the humus into carbon dioxide and soluble nitrogen compounds, as well as the unlocking of the insoluble mineral plant food present in the soils, was said to be stimulated to a great extent. However, most of these preparations have been very short-lived due to their inability to produce the results claimed for them. On the basis of properly controlled tests, none of them have been found to be worthy of recommendation.

[1] Contribution from Office of Soil-bacteriology and Plant-nutrition Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. Received for publication July 25, 1925.

[2] Associate Physiologist.

Concerning the latest invention named "Soilgro," the following data have been obtained. This material was first brought to our attention in 1924 when its preliminary exploitation was taking place in Canada. At present it is being sold in the United States.

Previous Tests With Soilgro

Through correspondence with Dr. Dan H. Jones, Agricultural College, Guelph, Ontario, Canada, the information was obtained that Soilgro was tested at the College farm on various crops, but none of the effects claimed had become evident. Later, the Canadian bacteriologists issued a joint statement regarding their adverse opinions on the merits of materials such as Soilgro. A chemical test of this preparation by the Massachusetts experiment station[3] showed it to be worth about 1 cent in fertilizing value. Practically the same chemical results were obtained at the Connecticut experiment station,[4] and tests on grasses and lettuce there failed to give any evidence in favor of the use of this material.

Description of Soilgro and Directions For Use

Soilgro is shipped in cardboard packages containing one or four units of material. Each unit is marked with the date beyond which the material will not be of value. The packages contain two 1-gallon cans of similar rectangular shape, one green in color, holding the bacteria, the other, brown, containing the "bacteria food."

The liquid is light brown in color, smelling somewhat like a weak rain water solution of manure. In this respect, it resembles Nitrobacter Soil Vaccine which was recently on the market and which was found to be practically worthless. The bacterial food consists of sandy

soil mixed with rotted manure, containing about 40% moisture. Plant stems and balls of manure covered with a white substance, presumably lime, were noticeable. The material is not homogeneous.

[3] Haskins, H. D. Chemical facts about Soilgro. In Rural New Yorker, 84:831. 1925.

[4] Morgan, M. F. Some experiments with "Soilgro." In Rural New Yorker, 84:970. 1925.

The directions accompanying Soilgro advise adding 4 gallons of rainwater to the furnished ingredients and incubating for five days in a warm place. Soilgro prepared according to directions is advised for use on lawns which are already growing, in which case the material properly diluted is sprinkled on under favorable conditions of cloudiness and rainfall, or the grass seed to be planted is moistened with the material undiluted. Other seed are soaked from 3 to 12 hours, the first time applying to soft seed like peas and beans, the second to hard seed such as radishes. Its use is also advocated on hardy perennials, house plants, and trees.

The samples of Soilgro used in the tests reported were obtained as follows: Sample No. 1 by direct purchase from the Soilgro Products Company, New York City; sample No. 2 by donation through the courtesy of Mr. Davieson Power, president of the company; and sample No. 3 by purchase through an agent at Philadelphia, Pennsylvania.

Numbers of Bacteria In Soilgro

The literature accompanying Soilgro states that the mixture contains legume bacteria, nitrogen fixing, ammonifying, nitrifying, and decomposition bacteria.

In determining the numbers (Table 1) of bacteria, beef agar was used for ammonifying organisms, soil extract agar for the general soil organisms, and cellulose agar for the decomposition bacteria. Potting soil and rotted manure were run separately for a comparative purpose.

TABLE 1.—Numbers of bacteria per gram grown from Soilgro, Soilgro mixture, and other preparations on three different media.

Material	Beef agar	Soil extract agar	Cellulose agar
Soilgro, Sample No. 1	2,900,000	393,000	186
Soilgro, Sample No. 2	1,520,000	1,100,000	
Soilgro, Sample No. 3	800,000	530,000	
Bacterial food, Sample No. 1	127,000,000	142,000,000	14,500
Rotted manure	370,000,000	327,000,000	430,000
Potting soil	35,000,000	69,000,000	230,000
Soilgro mixture, Sample No. 1	2,000,000	500,000	
Soilgro mixture, Sample No. 2	2,445,000		
Soilgro mixture, Sample No. 3	1,020,000		
Bacterial food mixture, Sample No. 1	2,000,000	880,000	
Rotted manure mixture	5,500,000	2,240,000	
Potting soil mixture	9,000,000	1,720,000	

1050 Numbers of bacteria were calculated without reference to the moisture content of the soil, manure, and bacterial food. The mixtures of soil, manure, and bacterial food were prepared similarly to Soilgro.

The figures indicate that there are considerably more bacteria in the bacterial food, in ordinary potting soil, and in rotted manure than in the Soilgro liquid, either before or after preparation according to directions.

Character of the Bacteria

The bacteria found in Soilgro and Soilgro mixture did not differ materially from those found in the soil which was plated at the same time on beef and soil extract agar. Typical Azotobacter films did not develop in mannite solutions. Colonies occurred on soil extract agar which resembled *B. radicicola*, but absolute proof of the presence or absence of certain strains of this organism is given in Table 2 which is a compilation of data from vegetative tests made under controlled conditions with potting soil and with Soilgro.

TABLE 2.—Strains of legume bacteria found in Soilgro.[a]

Inoculation	Nodule production on				
	Alfalfa	Red Clover	Cowpea	Soybean	Vetch
Potting soil	+	+	—	+	+
Bacterial food, Sample No. 1	—	+	—	—	+
Soilgro, Sample No. 1	+	+	—	—	—
Soilgro, Sample No. 2	+	—	—	—	+
Soilgro, Sample No. 3	+	+	—	—	—

[a] + indicates the organisms were found.
— that they were not found.

Nitrogen Fixation and Transformation

By the use of the standard methods of the Laboratory of Soil Bacteriology for the determination of the power of a culture to assimilate and transform nitrogen, tests[5] were made with the undiluted original samples of Soilgro bacteria, with the mixtures prepared according to directions, and with the bacterial food, as well as with potting soil for comparison. The incubation periods for the fixations and transformations were 10 and 20 days, respectively. The results are given in Tables 3 and 4.

[5] Nitrogen analyses were made by Klare S. Markley.

TABLE 3.—Nitrogen assimilation by soil and Soilgro.

Inoculum	Nitrogen in medium and inoculum mgs.	Nitrogen after 10 days' incubation mgs.	Net gain in nitrogen mgs.
5 grams of potting soil	11.8	14.9	3.10
5 grams bacterial food, Sample No. 1	14.0	15.6	1.60
5 cc. Soilgro, Sample No. 1	3.8	3.9	0.10
5 cc. Soilgro, Sample No. 2	1.1	1.7	0.60
5 cc. Soilgro, Sample No. 3	1.1	3.3	2.20
5 cc. Soilgro mixture, Sample No. 2	1.8	2.2	0.40
5 cc. Soilgro mixture, Sample No. 3	2.1	2.5	0.40

TABLE 4.—Transformation of ammonium salts to nitrate by soil and Soilgro.

Inoculum	Type of media	Nitrogen as nitrate in medium and inoculum mgs.	Nitrogen as nitrate after 20 days' incubation mgs.	Net production of nitrogen as nitrate mgs.
5 grams of potting soil	Liquid 50 cc. $(NH_4)_2HPO_4$	1.40	7.50	6.1
5 grams bacterial food, Sample No. 1	Liquid 50 cc. $(NH_4)_2HPO_4$	1.15	3.75	2.6
5 cc. Soilgro, Sample No. 1	Liquid 50 cc. $(NH_4)_2HPO_4$	1.60	5.80	4.2
5 cc. Soilgro, Sample No. 2	Soil 50 grams $(NH_4)_2SO_4$	9.0	10.6	1.6
5 cc. Soilgro, Sample No. 3	Soil 50 grams $(NH_4)_2SO_4$	9.0	11.0	2.0
5 cc. Soilgro mixture, Sample No. 2	Soil 50 grams $(NH_4)_2SO_4$	9.0	9.6	0.6
5 cc. Soilgro mixture, Sample No. 3	Soil 50 grams $(NH_4)_2SO_4$	9.0	9.6	0.6

A Vegetative Test With Radishes

Radish seed were soaked in the prepared solution of Soilgro bacterial food, soil, and manure for 12 hours, and then duplicate boxes were planted in uniform soil, 100 properly spaced seed to each box. The result from the first harvest of marketable radishes is given in Table 5.

TABLE 5.—First crop of marketable radishes from seed treated with Soilgro and other mixtures.

Treatment	Number of radishes of suitable size		Total weight in grams, including tops		Average weight in grams per radish
	Box 1	Box 2	Box 1	Box 2	
None	22	33	132	224	6.47
Soilgro mixture, Sample No. 1	30	34	190	244	6.78
Bacterial food mixture, Sample No. 1	30	33	213	242	7.22
Rotted manure mixture	26	32	189	231	7.24
Potting soil mixture	32	27	213	199	7.00

The small differences observed do not indicate that the Soilgro preparations have exerted any beneficial effect
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1051 upon the development of the test plants. This finding is confirmed by the data presented in Table 6.

TABLE 6.—Total crop of radishes from seed treated with Soilgro and other mixtures.

Treatment	Numbers		Total weight in grams, including tops		Average weight in grams per radish
	Box 1	Box 2	Box 1	Box 2	
None	82	82	519	599	6.82
Soilgro mixture, Sample No. 1	80	77	525	554	6.87
Bacterial food, Sample No. 1	73	81	518	542	6.88
Rotted manure mixture	81	71	564	536	7.24
Potting soil mixture	74	71	393	519	6.29

The figures given in the "numbers" column of Table 6 show no great variations in germination and also indicate that the soaking of the seed has not been of advantage in this case.

A Test With Garden Peas

Peas were soaked three hours in the Soilgro mixture and in other mixtures for comparison. They were planted in the field on a rather heavy clay soil. All seed germinated about the same time. Doves and pigeons dug up some of the seed, thereby making it necessary for comparative purposes to calculate the crop on the basis of the weight per plant. Unfortunately, dry weather interfered with the development of this crop. The results are given in Table 7.

TABLE 7.—Crop of peas from plats with various treatments.

Treatment	Number of plants	Total weight in grams of peas in pods	Average weight in grams of peas per plant
Soilgro mixture, Sample No. 1.....	257	363	1.41
Bacterial food mixture, Sample No. 1	278	547	1.96
Rotted manure mixture.....	279	564	2.02
Potting soil mixture.....	278	565	2.03

A Test On Lawns

The treatment of lawn grass with Soilgro has been tried on the grass garden at the Arlington Farm by the Office of Forage Crop Investigations. The plats treated with So gro and bacterial food exhibit a superficially noticeable inferiority to the plats treated with ammonium sulfate.

In another experiment, eight plats of 10 feet by 10 feet each were treated with the preparations heretofore mentioned in duplicate in accordance with the instructions. Each application was made under favorable conditions. At no time during the period following treatment were any superficial differences between the plats noted. As

a quantitative index of the effects of the treatments, the cuttings of the grass from the various plats were dried and weighed. One cutting was made before the first application and the other 26 and 12 days, respectively, after the first and second application of material. Only one application of the manure mixture was made. The results are given in Table 8.

TABLE 8.—Dry weights of grass grown on plats treated with Soilgro and other preparations.

Plat	Treatment	Weight of crop in grams before treatment	Average in grams	Weight of crop in grams after two treatments	Average in grams
2	Soilgro mixture,	403	306	293	254
5	Sample No. 1	208		214	
3	Bacterial food mixture,	226	297	320	347
6	Sample No. 1	367		373	
1	Rotted manure	280	207	292	268
8	mixture	134		244	
4	Potting soil mixture	182	268	238	301
7		354		364	

Summary

1. The total numbers of bacteria in the Soilgro cultures tested were found to be much lower than those in the so-called bacterial food accompanying the liquid cultures. Soil and manure samples plated for comparison gave higher figures.

2. The numbers of ammonifying and cellulose destroying bacteria were also larger in bacterial food, soil, and manure samples than in the Soilgro cultures. The presence of Azotobacter in the culture was not indicated by the tests made. Certain strains of legume bacteria were found in Soilgro, but in less number than in the potting soil used for comparison.

3. Nitrogen fixation by potting soil was superior to the fixation by a Soilgro culture, and the mixtures of Soilgro gave a lower fixation in two cases than the culture from which they were made.

4. The nitrification of ammonium salts in liquid and in soil cultures was better by potting soil than by any of the Soilgro preparations.

5. Soilgro showed no superiority in the production of radishes, either in the stimulation of germination, earliness of crop, or weight of crop collectively or individually. Tests made with peas were likewise negative.

6. Two tests on lawns did not produce results favorable to Soilgro. Quantitative tests on cuttings made from treated plats bore out the results of superficial examination.

1052 DEFENDANT'S EXHIBIT 25-A,

(Offered but not received in evidence.)

Canada

Division of Bacteriology and Dairy Research — Science Service

DEPARTMENT OF AGRICULTURE
Central Experimental Farm
Ottawa

Dr. A. W. Hofer, May 22, 1945.
Division of Bacteriology,
New York Agricultural Experiment Station,
Geneva, N. Y.

Dear Dr. Hofer:

I have your letter of May 18th, regarding the practicability of preparing composite culture inoculants, that is those which are able to cause nodulation on more than one of the cross-inoculation groups.

The matter is one on which there are surprisingly few data available in the literature, as far as I have been able to judge. Fred, Baldwin and McCoy have little to say on this question, and what they do say suggests that the disadvantages out-weigh the advantages. Wilson's book on Symbiotic Nitrogen Fixation also emphasizes the scarcity of data, while Bond (J. Bact. 36: 306) gives little in the way of supporting data in recommending composite cultures.

During the course of the last seven or eight years we have had occasion to check a few composite cultures for nodulation. Out of five tests we found that in four cases nodulation on red clover was considerably less satisfactory than on alfalfa plants, and in one case the reverse was true. However, I do not think that the differences in effectiveness noted provide any satisfactory evidence either for or against the hypothesis of antagonism between different species of Rhizobia, because we have no data on the use of the same cultures singly. Single cultures might or might not have given the same results. The above tests to which I refer had to do with the percentages of plants nodulated when the cultures were used as directed. No information was obtained as to nitrogen-fixation.

Regretting we have no further data on this important practical problem, I am,

Yours sincerely,
(signed) A. G. Lochhead,
A. G. Lochhead,
Dominion Agricultural Bacteriologist.

AGL/F

1057 DEFENDANT'S EXHIBIT 25-B

(Offered but not received in evidence.)

Division of Bacteriology

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION
Cornell University
Geneva, New York
A. J. Heinicke, Director

Geneva, N. Y., June 7, 1945

Dr. Alvin Hofer,
Agricultural Experiment Station,
Geneva, New York.

Dear Dr. Hofer:

I do not know of any data showing that one kind of legume nodule bacteria has an inhibitory effect on another. It is true that the earliest commercial inoculants contained only one variety each, but this seems to have been due to two circumstances only: first, the bacteriologist never feels that it is wise, in the laboratory, to use anything but pure cultures, and accordingly the advice given to the industry (until subsequent study showed it to be unnecessary) was to furnish the farmers with pure cultures; second, to avoid confusion on the part of the farmer, it seemed best to mark each inoculant as adapted to the one kind of legume on which it worked *best*. As early as 1931, however, commercial producers of these inoculants began putting mixed cultures on the market, and the success of these mixed cultures, in practice, seems to have disproved any possible claim that inhibition occurs when such varieties are combined in one culture.

Furthermore, we know that in many soils nodules are produced on the roots of several different kinds of legumes; this is absolute proof that the several varieties of nodule bacteria live together in soil without harming each other. There is no conceivable reason why they cannot do so in inoculants also.

Please note that I say "varieties" not "species". I use the former term, because students of this group are uncertain whether there is more than one species of *Rhizobium*, and in case there is more than one whether there are many or just two or three.

Sincerely,

(signed) H. J. Conn.
H. J. Conn.

HJC:bb

1056 DEFENDANT'S EXHIBIT 25-C.

(Offered but not received in evidence.)

Department of Bacteriology

THE PENNSYLVANIA STATE COLLEGE

School of Agriculture

State College, Pennsylvania

June 8, 1945

Dr. A. W. Hofer,
New York State Agricultural Experiment Station,
Cornell University,
Geneva, New York.

Dear Dr. Hofer:

I do not agree with the belief that one species of Rhizobium may exert a definite inhibitory effect upon another species of the genus. I do agree with the belief that it is not wise to cultivate two or more species together as one may so outgrow the other or others that only the rank grower will be present in significant numbers. This holds for any similar mixture of closely related organisms as for example mixtures of *E. coli* and *A. aerogenes.*

In so far as mixing two species of Rhizobium after growth is concerned, as for example might easily be done in humus preparations, it is my considered opinion that no adverse effects will take place and if used within a reasonable length of time such a mixture should be effective for all cross-inoculation groups concerned. I am opposed to the sale of such preparations as a rule because when one is inoculating soybean seed for example there would be no benefit in paying for another group which would not be useful.

Sincerely,

(signed) J. J. Reid,
J. J. Reid,
Associate Professor.

JJR:au

1053 DEFENDANT'S EXHIBIT 25-D.

(Offered but not received in evidence.)

College of Agriculture Department of Soils

UNIVERSITY OF MISSOURI
Columbia

May 29, 1945

Dr. A. W. Hofer
Division of Bacteriology
Agricultural Experiment Station
Geneva, New York

Dear Dr. Hofer:

We have your letter of the 18th inquirying about the possibility of using composite cultures and cross inoculating groups for seed treatment.

In our own experience we have found the cultures from one plant group serving very effectively when used on others in the cross inoculation classification as we now know it. The specificity of a culture, grown in the laboratory, on the plant out in the soil is a matter which seems to have been strained a bit in our desires to work out so many classifications. The work of Dr. Wilson has pointed forcefully to the fact that cross inoculations are wider in Nature than we are wont to believe.

In our own studies, the physiology of the plant has given it a decided prominence in determining whether it is inoculated or not. The particular kind of bacteria has had so much attention and so little attention has gone to the nutritional level of the plant that I am inclined to believe that we have emphasized our bacterial cultures too much and that the fertility of the soil into which these cultures are put has been emphasized altogether too little.

When different microorganisms live in the soil as a mixture, the matter of their living together in a culture deserves the belief that they get along together. This whole matter of excessive emphasis on the culture as to its specificity for one plant species is mainly a matter of the conditions of the laboratory. If we have the micro-organisms truly growing, only small numbers of them are needed to be effective on the plant if the plant conditions are favorable.

Yours very truly,
Wm. A. Albrecht,
Chairman,
Department of Soils.

1054 DEFENDANT'S EXHIBIT 25-E.

(Offered but not received in evidence.)

Department of Agronomy

University of Illinois
COLLEGE OF AGRICULTURE
Agricultural Experiment Station
Urbana, Illinois

May 26, 1945

Dr. A. W. Hofer,
Division of Bacteriology,
New York Agricultural Experiment Station,
Cornell University,
Geneva, New York.

Dear Doctor Hofer:

Our studies have not proved that one species of legume nodule bacteria has an inhibitory effect on other species. Because we have not studied all strains of Rhizobium leguminosarum it is not possible to state what effect "certain strains of certain species of the Rhizobia have on each other".

Very truly yours,
O. H. Sears,
Chief, Soil Biology.

OHS:pk

1058 DEFENDANT'S EXHIBIT 25-F.

(Offered but not received in evidence.)

State of New Jersey
AGRICULTURAL EXPERIMENT STATION
William H. Martin, Director
New Brunswick, N. J.

May 21, 1945

Dr. A. W. Hofer,
Div. of Bacteriology,
N. Y. Agr. Exp. Station,
Geneva, New York.

Dear Dr. Hofer:

I am in receipt of your letter of May 18 in regard to the lawsuit involving the two inoculant companies. I am very much surprised to learn that a company should make statements in regard to the effect of one legume organism upon another merely because the two exist in mixed culture. As far as our present knowledge is concerned, we have no evidence whatsoever that such an effect is taking place. This is sheer speculation and is not based upon either experimental evidence or upon practical observation.

There is no doubt that under natural conditions certain organisms have an inhibitory effect upon the growth of others, but these phenomena are exceptions rather than the rule. Although the possibility is not excluded that certain soil organisms may have some injurious effects upon legume bacteria, the possibility of the injurious effect of one legume organism upon another is, as I indicated above, rather speculative. One can even suggest that possibly one legume organism has a *favorable*, rather than injurious, effect upon another since such phenomena are quite abundant in nature.

Hoping that this information will be of interest to you, I am

Sincerely yours,
(sgd) Selman A. Waksman,
Microbiologist.

SAW:vab

1055 DEFENDANT'S EXHIBIT 25-G.

(Offered but not received in evidence.)

Soils Section Department of Ogronomy
THE STATE COLLEGE OF WASHINGTON
Pullman, Washington

College of Agriculture and
Agricultural Experiment Stations

May 23, 1945

Dr. Alvin W. Hofer,
Division of Bacteriology,
New York Agricultural Experiment Station,
Geneva, New York.

Dear Dr. Hofer:

With reference to your letter of May 18 I regret to state that we have made no exhaustive studies of the inhibitory effect of certain species of legume nodule bacteria on other species or cultures of the legume nodule bacteria when grown together. Judged from the results of the investigational work we have conducted along this line we have no reason for believing that one species of the legume nodule bacteria exerts significant inhibitory effects on other species of this group when grown together.

It is true that some species of bacteria in this group make more vigorous growth than others when grown separately on identical culture media, and it might be expected that this will occur also when they are grown together on the same culture medium.

Very truly yours,
(sgd) S. C. Vandecaveye,
Professor of Soils.

SCV:je

1059 DEFENDANT'S EXHIBIT 25-H.

(Offered but not received in evidence.)

HANSEN INOCULATOR CO.
(Hansen's Inoculator)
Trademark
808 North Lincoln Avenue
Urbana, Illinois

May 23, 1945

Dr. Alvin W. Hofer,
Division of Bacteriology,
N. Y. State Agr. Exp. Station,
Geneva, New York.

Dear Sir:

Replying to your letter of May 18, I have numbered the sentences in the patent referred to and given my reply immediately below.

1. "It has heretofore been considered impracticable to prepare a composite culture inoculant containing organisms which will cause nodulation on more than one of the cross-inoculation groups."

A. This is not true. As a matter of fact, several companies, including ourselves, were manufacturing and selling garden packets containing at least three groups for some years before the patent was taken out.

2. "This has not been done because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen fixation by the plant and the organism was inhibited or even prevented."

A. This is not true. No such inhibitory effect has ever been proved. No such inhibitory effect occurs in soils. For example, alfalfa bacteria, clover bacteria and soybean bacteria will live in the same soil without inhibitory effect one to the other. Reduction
1060 in numbers does occur and the chief factor responsible is very generally agreed to be the soil acidity. Other factors are fertility level of the soil, moisture conditions and temperature (alternate freezing and thawing). There is no reason to believe that the nodule bacteria behave differently in a culture

than in ordinary soils. After all, the finely ground, kiln dried peat most commonly used as a base in preparing commercial cultures is an organic soil.

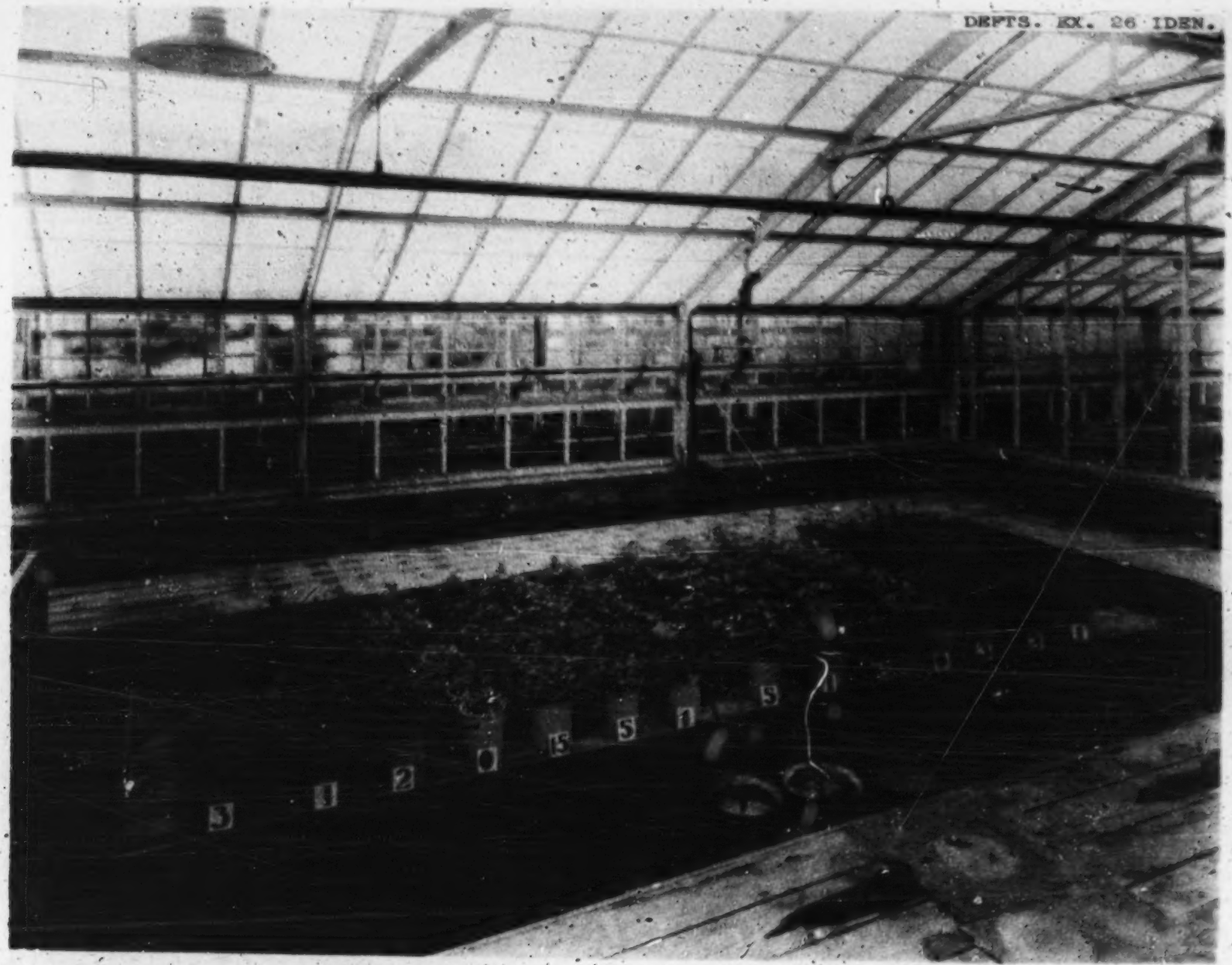
3. "For this reason, it has been considered desirable to use a culture containing a single species of organism rather than one containing a mixture."

A. The reason single species cultures have been considered most desirable for cultures for field seeds is that the peat base will only take up so much moisture (35 to 40 per cent), and to get enough bacteria into a given amount of peat base only one species is used. If you use three species, the numbers of bacteria of each group is reduced to one-third. If you triple the size of the package to get the proper numbers for each group, the farmer gets into trouble in applying so much peat-base inoculant to a given amount of seed. Production costs also enter in the picture; two-thirds of the contents of each package is pure waste, since in almost every case the contents of the package is used on one kind of seed. The objections to the use of composite cultures for field seeds does not apply to garden seeds. In garden packets the amount of inoculant is much greater in proportion to the amount of seed; and garden seeds are very generally hand planted, so the difficulty in seeding does not occur.

1061 Finally, it is my considered judgment that the only advantage in composite cultures for field seeds lies in the reduction of returns by dealers. If the dealer fails to sell the package for alfalfa or sweet clover, he may be able to sell it for clovers; if he fails to sell it for either of these groups, he may sell it for soybeans. The advantage is to the dealer and not to the farmer who is the ultimate consumer. This fact becomes obvious when one examines the advertising by makers of composite cultures in seed trade journals.

Yours very truly,
(sgd) Roy Hansen.

RH:agt


DEPTS. EX. 26 IDEN.
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DEFTS. EX. 27 IDEN.

699-999

DEFTS. EX. 28 IDEN
668-669

1065 DEFENDANT'S EXHIBIT 29.

CLOVER PLANTS

WEIGHTS EXPRESSED IN GRAMS

	a	b	c	d	e	f	g	h
1 Tops	15.0	12.9	12.2	11.6	12.4	13.2	15.7	11.0
1 Roots	12.2	6.9	7.3	7.3	9.0	8.2	11.9	9.1
1 Total	27.2	19.8	19.5	18.9	21.4	21.4	27.6	20.1
2 Tops	23.9	13.6	10.5	10.8	12.8	12.3	12.7	8.3
2 Roots	8.9	9.2	5.8	5.1	8.0	7.8	7.7	10.0
2 Total	32.8	22.8	16.3	15.9	20.8	20.1	20.4	18.3
3 Tops	18.9	15.7	12.8	9.3	16.0	9.3	16.4	13.6
3 Roots	12.2	9.9	8.6	17.3	9.4	6.5	9.7	9.1
3 Total	31.1	25.6	21.4	26.6	25.4	15.8	26.1	22.7
4 Tops	19.3	6.7	10.6	15.0	16.2	10.5	12.7	16.0
4 Roots	13.6	12.7	5.6	8.5	13.4	4.7	6.2	10.5
4 Total	32.9	19.4	16.2	23.5	29.6	15.2	18.9	26.5
5 Tops	16.4	18.0	12.9	15.5	13.6	11.2	11.1	12.8
5 Roots	12.3	9.2	6.0	7.7	8.6	9.1	8.7	13.3
5 Total	28.7	27.2	18.9	23.2	22.2	20.3	19.8	26.1
1–15 Tops	22.9	14.3	19.3	12.5	10.4	17.1	14.0	14.5
1–15 Roots	11.1	8.2	9.7	6.7	5.5	13.3	7.2	11.1
1–15 Total	34.0	22.5	29.0	19.2	15.9	30.4	21.2	25.6
0 Tops	1.0	0.4	0.5	0.5				
0 Roots	2.5	1.9	2.0	2.5				
0 Total	3.5	2.3	2.5	3.0				

CLOVER PLANTS

WEIGHTS EXPRESSED IN GRAMS

Total	Average	% N	Total N % 8 pots	Total
104.0	13.0	3.22	3.35	
71.9	9.0	2.47	1.77	
	22.0		5.12	5.12
104.9	13.1	3.33	3.49	
62.5	7.8	2.28	1.43	
	20.9		4.92	4.92
112.0	14.0	3.15	3.53	
82.7	10.34	2.20	1.82	
	24.34		5.35	5.35
107.0	13.8	3.22	3.45	
75.2	9.4	2.39	1.80	
	23.2		5.25	5.25
111.5	13.9	3.42	3.81	
74.9	9.36	2.33	1.75	
	23.26		5.56	5.56
125.0	15.6	3.42	4.27	
72.8	9.1	2.22	1.62	
	24.7		5.89	5.89
2.4	0.6			
8.9	2.2	0.97	0.218	0.218
11.3	2.8			

1066 DEFENDANT'S EXHIBIT 30.

COMPARISON OF TYPES OF CULTURES BASED UPON TESTS MADE IN NEW YORK STATE

	Cultures for one plant group		Garden cultures	
Year	Number tested	Number Unsatisfactory	Number Tested	Number Unsatisfactory
1933	51	7	7	4
1934	64	6	11	3
1935	59	14	6	1
1936	98	4	10	1
1937	90	2	7	2
1938	99	1	11	1
1939	60	1	1	0
1940	57	1	4	0
1941	56	1	8	0
1942	68	3	5	0
1943	71	0	6	0
1944	54	1	8	0

One company at least produced good garden cultures so long as these were tested.

With the exception of one brand that had two unsatisfactory garden cultures in one year, the quality of these has been good.

1067 DEFENDANT'S EXHIBIT 31.

YIELD OF LIMA BEANS FROM TWO TYPES OF INOCULANTS

Type of culture	Total Yield in Grams							
	1941			Aver-	1942			Aver-
	Row 1	Row 2	Row 3	age	Row 1	Row 2	Row 3	age
None	733	891	1309	968	1945	1190	2034	1723
Lima Bean	1375	1603	1932	1637	1530	2460	1685	1892
Lima Bean	1385	1549	1246	1397	1914	1945	2142	2000
Mixed	2414	2255	2410	2293	2851	3203	2135	2730
Mixed	2323	1183	1161	1556	2925	2304	1777	2335
Mixed					1964	2217	1575	1919

The average yield of the two mixed cultures tested in 1941 is found to be 1924 grams per row, as contrasted to the 1517 grams of the lima bean cultures; in 1942, it is 2328 grams for the mixed culture, and 1946 grams for the lima bean cultures. These results are similar to those obtained in 1942 with beans. Thus, in every test so far made, the culture containing nodule bacteria for more than one group of legumes have outyielded those made for a single group. These results indicate that nitrogen fixation by bacteria from mixed cultures can be as good as that of bacteria from the more traditional type of inoculant. If the various groups of nodule bacteria packed together in one container had any effect on each other in these instances, this would appear to have been favorable.

AGRICULTURAL BULLETIN

Published monthly by the Department of Agriculture and Markets of the State of New York

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ALBANY, N. Y. NOVEMBER, 1934

Bulletin 292

Report of Analyses of Samples of Seed Collected in New York State July 1, 1933, to June 30, 1934

Defendant's No. 32
F.T.C.

Also

Report on Legume Inoculant Tests Made in 1934

(Pages 68 to 72 inclusive)

LEGUME INOCULANT TESTS FOR 1934

This report includes the results of tests upon legume inoculants purchased during 1934. As in previous years, these were secured by an inspector of the Bureau of Food Control, of the Department of Agriculture and Markets, in the seed stores and other places of sale in the State. The total number was 75, all of which were tested in the laboratories and greenhouses of the New York State Agricultural Experiment Station at Geneva.

Tests for Legume Inoculants

There are, in general, two types of legume inoculants, and these are tested by different methods. One type consists of such materials as agar and sand which carry the bacteria in pure culture; the other, of unsterilized peat or soil to which the bacteria are added.

Methods: Those cultures which were known to carry the bacteria in pure culture were tested by both laboratory and greenhouse methods. In the laboratory, bacterial counts were made by use of culture plates, and tests to detect presence of foreign bacteria were carried out in every case. If foreign bacteria were present, the estimation of numbers was not carried out. In the other type of culture, where foreign bacteria were always present, plate counts could not be made, in spite of the fact that such a count is of valuable assistance in the determination of the quality of a culture.

Greenhouse tests were the same for both types of culture, and were carried out in the same way as last year, with the exception that only two dilutions were used for the inoculation of the plants. These consisted of the one recommended by the manufacturer on the label, and another consisting of $\frac{1}{10}$ that quantity of inoculum. Some of the tests, however, were carried out on two host plants. Thus, in the case of alfalfa inoculants, tests were carried out on alfalfa and sweet clover. In the clover group, alsike clover was the second plant used. The red clover and alfalfa were grown in half-gallon pots, as in other years; the sweet clover and alsike clover were grown in tumblers which were kept covered the first two weeks with the top of a culture plate of the type used in the laboratory tests. This protected the plants against contamination with bacteria from other pots in the greenhouse and thus proved a distinct advantage.

Results: The 1934 tests are reported in detail in Table I. It will be seen that although great improvement in quality has been made, there is still an occasional unsatisfactory culture. While the presence of unsatisfactory cultures is an indication that a change in the method of manufacture is necessary, it is, nevertheless, extremely difficult for a manufacturer to produce inoculants that are reliable in 100 per cent of the cases. For this reason, a product should not be judged too severely upon the basis of one inferior sample.

Tests of Non-specific Inoculants

Methods: In addition to the usual tests, an examination was made of a culture which was claimed to be useful for inoculation of a number of crops, including non-legumes. For this test two poor soils were selected, and two crops, corn and soybeans, were grown. Poor soils were used because any response would be greater in a poor soil than in a rich one. Upon each soil, and with each crop, five treatments were used. In every case, extreme care was used in following the directions on the label.

The first treatment consisted of soaking the seeds in ordinary water, because if the growth were increased it might easily be the result of rapid germination due to soaking. The second set in each case was soaked in a 1:5 solution of the inoculant. The third was untreated. The fourth was treated the same as the second with the exception that the inoculant was added to the roots also. The fifth set was treated exactly like the fourth except that the inoculant was sterilized before application to the seeds and plant roots. The plants were watered with ordinary tap water as it was needed, and when the plants had reached the limit of their growth, they were carefully removed from the sand, dried, and weighed.

Results: When the dried plants were weighed, it was found that there were no great differences between the series, except for number five. In one or two cases, a distinct increase in growth occurred in this series, but since every application of the inoculants to number five had been sterilized previously, the results can hardly be ascribed to the bacteria in the culture. For these reasons, permission to sell this brand in the State was refused.

Conclusions

The quality of legume inoculants on sale in this State is reaching the point at which it should be maintained. There is apparently still some difficulty in the production of inoculants of uniformly high quality, but under the stimulus of the tests, and by cooperation between manufacturers and those engaged in the tests, the situation in New York State has shown steady improvement. In addition to the tests of products now on sale in the State, a close watch is necessary for protection against new untested products which may be introduced.

In view of the fact that few of the other States exercise any control whatever over legume inoculants, farmers are urged to buy these products only through dealers in New York State.

TABLE 1—RESULTS OF LEGUME CULTURE TESTS, 1934

Sample number	Brand	Group	Bacterial count*	Nodule Formation†		Conclusions
				With 1/10 the recommended inoculum	With the recommended inoculum	
148	Nod-O-Gen	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
157	Nod-O-Gen	Alfalfa	S	S	S	Satisfactory
		Sweet clover		I	S	Satisfactory
161	Nod-O-Gen	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
175	Nod-O-Gen	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
178	Nod-O-Gen	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
149	Nod-O-Gen	Red clover	S	S	S	Satisfactory
		Alsike clover		U	S	Satisfactory
156	Nod-O-Gen	Red clover	S	S	S	Satisfactory
		Alsike clover		S	S	Satisfactory
160	Nod-O-Gen	Red clover	S	S	S	Satisfactory
		Alsike clover		S	S	Satisfactory
155	Nod-O-Gen	Pea	S	S	S	Satisfactory
162	Nod-O-Gen	Pea	S	S	S	Satisfactory
154	Nod-O-Gen	Soybean	S	S	S	Satisfactory
163	Nod-O-Gen	Soybean	S	S	S	Satisfactory
192	Nod-O-Gen	Bean	S	S	S	Satisfactory
193	Nod-O-Gen	Bean	S	S	S	Satisfactory
191	Nod-O-Gen	Pea	S	S	S	Satisfactory
		Bean		S	S	Satisfactory
		Lima bean		S	S	Satisfactory
194	Nod-O-Gen	Pea	S	S	S	Satisfactory
		Bean		S	S	Satisfactory
		Lima bean		S	S	Satisfactory
201	Nod-O-Gen	Cowpea	S	U	U	Unsatisfactory
135	G. L. F.	Alfalfa	U	U	U	Unsatisfactory
		Sweet clover		U	U	Unsatisfactory
144	G. L. F.	Alfalfa	P	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
153	G. L. F.‡	Alfalfa	S	I	I	Inconclusive
		Sweet clover		I	I	Inconclusive
167	G. L. F.‡	Alfalfa	U	U	U	Unsatisfactory
		Sweet clover		U	U	Unsatisfactory
303	G. L. F.	Alfalfa	U	U	U	Unsatisfactory
		Sweet clover		U	U	Unsatisfactory
158	G. L. F.	Red clover	S	S	S	Satisfactory
		Alsike clover		I	S	Satisfactory
166	G. L. F.	Red clover	P	S	S	Satisfactory
		Alsike clover		U	S	Satisfactory
186	G. L. F.‡	Red clover	S	S	S	Satisfactory
		Alsike clover		S	S	Satisfactory
197	G. L. F.‡	Red clover	S	U	S	Satisfactory
		Alsike clover		U	S	Satisfactory
190	G. L. F.‡	Red clover	S	S	S	Satisfactory
		Alsike clover		U	S	Satisfactory

TABLE 1 — RESULTS OF LEGUME CULTURE TESTS, 1934 — (*Continued*)

Sample number	Brand	Group	Bacterial count	Nodule Formation †		Conclusions
				With 1/10 the recommended inoculum	With the recommended inoculum	
202	G. L. F.	Red clover	U	U	U	Unsatisfactory
		Alsike clover		U	U	Unsatisfactory
152	G. L. F.	Pea	P	S	S	Satisfactory
190	G. L. F.‡	Pea	S	S	S	Satisfactory
196	G. L. F.‡	Pea	S	S	S	Satisfactory
173	G. L. F.‡	Soybean	S	S	S	Satisfactory
174	G. L. F.‡	Soybean	P	S	S	Satisfactory
200	G. L. F.‡	Soybean	S	S	S	Satisfactory
136	Nitragin	Alfalfa		S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
150	Nitragin	Alfalfa		S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
137	Nitragin	Red clover		U	S	Satisfactory
		Alsike clover		S	S	Satisfactory
151	Nitragin	Red clover		U	S	Satisfactory
		Alsike clover		S	S	Satisfactory
138	Nitragin	Pea		S	S	Satisfactory
176	Nitragin	Pea		S	S	Satisfactory
139	Nitragin	Soybean		S	S	Satisfactory
189	Nitragin	Soybean		S	S	Satisfactory
177	Nitragin	Pea		U	S	Satisfactory
		Bean		S	S	Satisfactory
		Lupine		S	S	Satisfactory
185	Nitragin	Pea		S	S	Satisfactory
		Bean		S	S	Satisfactory
		Lupine		S	S	Satisfactory
187	Nitragin	Pea		S	S	Satisfactory
		Bean		S	S	Satisfactory
		Lupine		S	S	Satisfactory
188	Nitragin	Lespedeza		S	S	Satisfactory
168	Nodule-Bacter	Alfalfa	S	U	S	Satisfactory
		Sweet clover		U	S	Satisfactory
179	Nodule-Bacter	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
169	Nodule-Bacter	Red clover	S	S	S	Satisfactory
		Alsike clover		S	S	Satisfactory
180	Nodule-Bacter	Red clover	S	S	S	Satisfactory
		Alsike clover		S	S	Satisfactory
170	Nodule-Bacter	Pea	S	S	S	Satisfactory
181	Nodule-Bacter	Pea	S	S	S	Satisfactory
172	Nodule-Bacter	Soybean	S	S	S	Satisfactory
183	Nodule-Bacter	Soybean	S	S	S	Satisfactory
171	Nodule-Bacter	Bean	S	S	S	Satisfactory
182	Nodule-Bacter	Bean	S	S	S	Satisfactory
184	Nodule-Bacter	Pea	U	U	U	Unsatisfactory
		Bean		I	I	Inconclusive
		Lima bean		U	U	Unsatisfactory
195	Nodule-Bacter	Pea	S	S	S	Satisfactory
		Bean		S	S	Satisfactory
		Lima bean		U	S	Satisfactory

TABLE I — RESULTS OF LEGUME CULTURE TESTS, 1934 — *(Concluded)*

Sample number	Brand	Group	Bacterial count *	Nodule Formation †		Conclusions
				With 1/10 the recommended inoculum	With the recommended inoculum	
132	Stimugerm	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
141	Stimugerm	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
145	Stimugerm	Alfalfa	S	S	S	Satisfactory
		Sweet clover		S	S	Satisfactory
140	Stimugerm	Red clover	P	U	S	Satisfactory
		Alsike clover		S	S	Satisfactory
198	Stimugerm	Red clover	S	S	S	Satisfactory
		Alsike clover		S	S	Satisfactory
142	Stimugerm	Pea	P	S	S	Satisfactory
146	Stimugerm	Pea	S	S	S	Satisfactory
133	Stimugerm	Soybean	S	S	S	Satisfactory
143	Stimugerm	Soybean	S	S	S	Satisfactory
147	Stimugerm	Soybean	S	S	S	Satisfactory
130	Stimugerm	Pea	P	I	U	Unsatisfactory
		Bean		I	S	Unsatisfactory
131	Stimugerm	Pea	P	I	S	Satisfactory
		Bean		I	S	Satisfactory
134a	McQueen's	Pea		U	S	Satisfactory
		Bean		S	S	Satisfactory
		Lupine		U	S	Satisfactory
134b	McQueen's	Pea		U	S	Satisfactory
		Bean		S	S	Satisfactory
		Lupine		U	S	Satisfactory
164	Strasburger & Siegel	Pea	U	U	S	Satisfactory
165	Strasburger & Siegel	Pea	U	U	U	Unsatisfactory
159	Berry's	Alfalfa		U	S	Satisfactory
		Sweet clover		S	S	Satisfactory

* Bacterial counts were made on pure cultures only; this explains the blank spaces in this column. The letter S (satisfactory) indicates that per pound of seed the culture contained over eight million organisms which were apparently root nodule bacteria, P (poor) that the count was between one and eight milljons, and U (unsatisfactory) that less than one million were present per pound of seed.

† S = Satisfactory
U = Unsatisfactory
I = Inconclusive

‡ These cultures were made by a new process.

Circular No. 137 (Revised)

Perry W. Wilson

New York State Agricultural Experiment Station

Geneva, N. Y.

SOIL INOCULANT SERVICE

In 1930, a law was adopted by the State of New York instructing Station to test for the Department of Agriculture and Markets ples of soil or plant inoculants sold or offered for sale in the te. Up to the present time these inoculants, with one exception,

FIG. 1.—THE STATION GREENHOUSES.
One of these houses is devoted to the testing of legume inoculants.

been cultures of bacteria intended for the inoculation of legume The Station has, accordingly, undertaken the work of testing e cultures, and the results of the tests are published at Albany lletins of the Department.

The official samples of legume inoculants are purchased by an pector of the Department of Agriculture and Markets at stores or ewhere they are offered for sale to the public. Tests are then e in the Station laboratories and greenhouses, to determine the bility of the cultures and to show whether the claims made by nufacturers are in any way misleading.

o far only one case has been found where the culture to be ered for sale was so obviously worthless that the Department of

Agriculture and Markets refused to permit registration. In this instance, the culture was a foul-smelling liquid offered for sale in an attractive-looking tin can. The manufacturers made claims as to its ability to inoculate not only legumes but almost any other kind of crop and to stimulate plants to reach maturity in a short period of time. The culture contained many bacteria, to be sure, as would a similar canful of liquid manure or any other decomposing liquid; but tests made at the Station failed to show justification for the manufacturer's claims. Accordingly, permission to register this product for sale in New York was refused and as a result there will be no high-pressure campaign to sell this material to farmers of this State.

The work of 1931, the first year that these tests were conducted, showed, however, that there were also a number of inferior legume inoculants on the New York market. It became apparent when tests were made that some of the cultures which sold in largest quantities were among the poorest on the market. In one instance, the company was a reliable concern which had been in business for many years but had specialized in the selling of other products. At a meeting of their representatives and Station workers, improvements were suggested. These have since been made, and the cultures produced by this company performed satisfactorily in the 1933 tests.

The other inferior samples were from a company which also is careful to maintain high standards. Studies have been made at this Station with the intention of finding what point in the process of manufacture needs to be corrected to bring all of this company's cultures to the proper level of quality. It is expected that the changes necessary to bring this about will be made shortly, so that the 1934 product will undoubtedly be of good quality.

Usually, the inferiority of cultures is due to unsatisfactory methods of production, but in other cases it occurs because the products are kept too long by the retailer before sale.

In general, three methods of improving the situation are available, namely, (1) refusal by the Department of Agriculture and Markets to register cultures for which entirely unreasonable claims are made, (2) securing the cooperation of manufacturers in improving their methods and in withdrawing old cultures from the market; and (3) educating farmers as to the greater value of fresh cultures. Usually the second method is best, but it is still necessary that farmers exer-

cise care to buy only such cultures that have not passed their date of expiration.

In addition to these activities, the Station has been carrying on experiments endeavoring to improve and to shorten the prevailing methods for testing legume cultures. Since the inoculants consist of tiny living germs, rapid chemical tests cannot be used to determine their identity and their numbers. The only reliable method at present available is the use of the culture with the plants for which it is recommended. This requires carefully controlled greenhouse experiments in which the culture is used to inoculate sterilized seeds growing in sterilized sand. Even this method has its drawbacks, as will be appreciated when it is understood that a gust of wind blowing a little dust from a properly inoculated pot to one in which an ineffective culture is being tested may spoil the results of the latter experiment. Improved methods are desired, both for greater reliability and greater speed. Definite progress in both respects has already been made, and as a result, further improvement and simplification is expected.

In these ways the Station is assisting the farmer in his efforts to purchase reliable inoculants for seed treatment. It is desired to enable him to purchase the cultures on their merits without danger of confusion by the advertising claims of the manufacturer. If he is misled by these claims and pays money for a worthless culture, he loses not only the small sum he pays for the inoculant, but also the much larger sum which he will lose from failing to get the expected improvement in his crops. This might show itself only in a slightly lowered yield, or it might consist in the loss of practically the entire crop; on some land, indeed, inoculation of a legume spells the difference between success and failure. In such cases, legume inoculation is cheap insurance against loss of seed, labor, and harvest; and to the individual farmer who follows this practice, a reliable inoculant is an absolute necessity.

At the present time, it is gratifying to be able to say that the situation with regard to legume inoculants has improved materially in this State. Whereas, in 1931 and 1932, the tests showed that a high proportion of the cultures were unsatisfactory, in 1933 they indicated a much smaller percentage as inferior. This last year the poor products were often found to be mixed cultures sold in small containers, recommended for garden purposes. Those intended for

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the inoculation of soybeans were also of low quality. Further tests are necessary to determine the seriousness of the situation and to learn what measures should be taken to correct it.

Close attention is always given to any new cultures that may be placed on sale in the State, that they be of such quality as to inoculate the plants for which they are recommended. Both seed dealers and farmers would do well to bear in mind, however, that the official inspection of legume inoculants applies only to cultures offered for sale within the State and not to cultures purchased outside its limits. For full protection of the law, therefore, the buyer should make his purchases of legume inoculants within the borders of New York. The results of the tests made in 1933 are published in the Agricultural Bulletin of the Department of Agriculture and Markets. Seed dealers can easily secure information from this source as to the reliability of the various brands. Farmers will perhaps find it advantageous to obtain information concerning cultures directly from the Station or the local Farm Bureau agent.

The names of cultures which have been tested and found reliable for field crops are as follows: G. L. F., Nitragin, Nodule-Bacter, Urbana Culture, Nod-O-Gen, Strasburger and Siegel, Scott's and Stimugerm. In the future, additional changes will be made in the approved list as new names are added or old ones dropped. Further information regarding inoculants or the provisions of the law may be obtained upon request to this Station.

January 1, 1934

H. J. CONN and A. W. HOFER

1078 DEFENDANT'S EXHIBIT 34.

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DEPARTMENT OF COMMERCE

United States Patent Office

To all persons to whom these presents shall come, Greeting:

THIS IS TO CERTIFY that the annexed is a true copy from the records of this office of the File and Contents, in the matter of the

Letters Patent of

Varley Sherman Bond, assignor to Kalo Inoculant Company,

Number 2,200,532, Granted May 14, 1940,

for

Improvement in Bacterial Inoculants for Leguminous Plants.

IN TESTIMONY WHEREOF I have hereunto set my hand and caused the seal of the Patent Office to be affixed at the City of Washington, this twenty-sixth day of May, in the year of our Lord one thousand nine hundred and forty-five and of the Independence of the United States of America the one hundred and sixty-ninth.

ATTEST:

D. E. WILSON
Chief of Division.

CONWAY P. COE,
Commissioner of Patents.

(Seal)

1079

NUMBER (Series of 1935) 226565 PATENT NO. 2200532

1938

Dated May 14, 1940

Div. 59 (Ex'r's Book) 121-9-071

Name VARLEY SHERMAN BOND,

Assor to Kalo Inoculant Company, of Quincy, Ill., a Corp. of Illinois.

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of QUINCY, State of ILLINOIS.

Invention BACTERIAL INOCULANT FOR LEGUMINOUS PLANTS

Parts of Application Filed

ORIGINAL	RENEWED
Application Filed Complete Aug. 24, 1938	.....
Petition, Specification, Oath, First Fee $30, } Aug. 24, 1938	.....
1 sheets Drawings,	.....
1 " Drgs. Mar. 11, 1940	.....
.....	.....
.....	.....
Examined and passed for Issue Apr. 1, 1940. P. W. Shepard Exr. Div. 59	Reexam'd and passed for Issue....., 193 Exr. Div.
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By Commissioner	By Commissioner
Final Fee $30 Apr. 13, 1940	Final Fee....., 193

Division of App. No. filed 19....

Attorney AMES, THIESS, OLSON & MECKLENBURGER, 77 West Washington St., Chicago, Ill.

Associate Attorney .....

.....

No. of Claims Allowed 14 Print Claims 1 & 10 in O.G. Class 71-7

Title as allowed Bacterial Inoculant for Leguminous Plants.

.....

1080

PETITION.

Aug.-24-38 037479 H Check 30.00

To the Commissioner of Patents:

Your Petitioner, Varley Sherman Bond, citizen of the United States, and a resident of Quincy in the County of Adams and State of Illinois, whose Post Office address is 1422 Maine Street, Quincy, Illinois, prays that letters patent may be granted him for the improvements in Bacterial Inoculant for Leguminous Plants set forth in the annexed specification.

And he hereby appoints the firm of AMES, THIESS, OLSON & MECKLENBURGER, consisting of Robert Lewis Ames, J. Bernhard Thiess, A. F. Mecklenburger, Arthur A. Olson, Willard L. Pollard, George W. Hansen, A. Arnold Brand, Thorley von Holst, Bertram Wm. Coltman, Albert H. Pendleton, and Carl Marx, 77 West Washington Street, Chicago, Illinois, his attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent and to transact all business in the Patent Office connected therewith.

Signed at Quincy in the County of Adams and State of Illinois, this 20th day of August, 1938.

Reg. No. 9622.

Varley Sherman Bond

(Inventor sign here, including first name in full)

TO ALL WHOM IT MAY CONCERN:

Be it known that I, Varley Sherman Bond a citizen of the United States, residing at Quincy in the County of Adams and State of Illinois, have invented new and useful improvements in BACTERIAL INOCULANT FOR LEGUMINOUS PLANTS, of which the following is a full, clear, concise and exact description, reference being had to the accompanying drawing forming a part of this specification.

1081 This invention relates to a bacterial culture and more specifically to a bacterial culture useful for the inoculation of the seeds of leguminous plants.

It is well known that certain plant species of the genus Leguminosae may under certain conditions fix nitrogen directly from the air and convert it to organic nitrogenous compounds. The mechanism of this nitrogen fixation is not understood at the present time, but it has been known for a number of years that it can only be carried out when the plant grows in symbiotic relationship with certain bacteria of the genus Rhizobia. Plants belonging to the genus Leguminosae which are capable of symbiotic relationships with the Rhizobia are peas, beans, alfalfa, red clover, white clover, vetch, lupines, and the like. The bacteria infect the roots of these plants in the soil and cause the formation of so-called nodules,

in which it is presumed that the nitrogen fixation in the plant occurs.

Other plants that are important in agriculture, such as the grasses and grains, are not able to fix nitrogen from the air. They depend entirely upon the combined nitrogen in the soil, such as nitrates and ammonium salts, for their nitrogen requirements. Consequently, the combined nitrogen in the soil becomes depleted after a series of crops consisting only of the non-nitrogen fixers have been grown therein. It has consequently become customary in many districts to practice crop rotation, whereby the plants that are capable of fixing nitrogen in the soil will enrich the soil with combined nitrogen, whereby nitrogenous nutrients are provided for a succeeding crop of non-nitrogen fixing plants. More recently it has become the practice to plant mixed crops, which comprise a mixture of the nitrogen fixing and the non-nitrogen fixing variety. Nitrogen is thus supplied to the soil in a combined form which may be readily utilized by the non-nitrogen fixing plants.

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Bacteria of the genus Rhizobia which are capable of infecting leguminous plants so that nitrogen may be fixed are widely spread in nature. They are not, however, universally present in soil and it has been found desirable to inoculate the seeds of leguminous plants with a culture of bacteria of the genus Rhizobia previous to the planting of the seeds in the field. This inoculation insures that micro-organisms of the correct species will be present in the soil whereby the leguminous plant may become infected. In the absence of such infection as pointed out above, no nitrogen fixation occurs within the plant. The inoculation of seeds is particularly desirable where it is desired to plant them in a soil that has not grown a plant of the same species for a number of years. In order to insure a maximum amount of nitrogen fixation and development of the leguminous plant, it is always desirable to inoculate the seeds previous to the planting thereof.

Figure 1 shows a root portion of a red clover plant bearing nodules A which were produced by infection with Rhizobium trifolii. This view was taken with

permission of the authors from a photograph reproduced in the monograph Root Nodule Bacteria and Leguminous Plants, by Fred, Baldwin and McCoy, University of Wisconsin Studies in Science, No. 5, 1932.

Fig. 2 shows a group of forty red clover plants after ten weeks' development in a sterile nitrogen-free sand culture which was not inoculated previous to the time of planting.

Fig. 3 shows a group of forty-one red clover plants of the same age and number grown in the same nitrogen-free substrata as those in Fig. 2, but wherein the seeds were inoculated with a culture of Rhizobium trifolii previous to planting.

Fig. 4 is the same as Fig. 3 but wherein the seeds were inoculated with a composite culture prepared in accordance with one embodiment of this invention.

It was discovered early that all species of the genus Rhizobia would not infect all of the plants of the genus Leguminosae to produce nodules wherein the nitrogen fixation presumably takes place. Thus certain species of bacteria are specific to certain species of Leguminosae. The group of plants that will be infected by a single species of bacteria has come to be known in the art as a cross-inoculation group. There are at least six well-recognized species of Rhizobium which will infect six fairly well-defined cross-inoculation groups of plants. Rhizobium meliloti will infect alfalfa and white or yellow sweet clovers; Rhizobium trifolii will infect red, white, crimson or mammoth clovers; Rhizobium leguminosarum will infect garden or field peas, vetch, and broad beans; Rhizobium phaseoli will infect garden or field beans; Rhizobium lupini will infect the lupines; and Rhizobium japonicum will infect the soy beans. The above groups are the most important in regard to inoculation of the seeds of field crops previous to sowing. There is a mixed group, including cow peas, lespedeza, peanuts, velvet beans, lima beans and crotalaria, which has not been well enough defined with respect to cross-inoculation to be assigned a definite terminology. This latter group, however, have been relatively unimportant for the

preparation of commercial bacterial inoculants for the seeds of leguminous plants.

A number of so-called commercial cultures of bacteria have appeared on the market from time to time which were purported to be useful for producing nodulation of the roots of the leguminous
1084 plants. Many of these cultures have been very effective and useful for the purpose for which they were to be employed. These cultures have appeared in a large variety of forms and contain a large number of viable strains of the desired organism. They have been distributed on suitable jelly bottle slants or in a wet or dry carrying material such as sand, soil, or charcoal.

A variety of methods have also been employed for producing the bacterial inoculant for commercial distribution. In the preparation of an inoculant or culture in accordance with one method the carrying media, such as humus or charcoal, may be inoculated with a suitable strain of the organism, and sugar, mineral salts, and water may be added with subsequent development of the species of organism therein. The resulting material is then packaged and distributed in any desired manner. This is known as the simple humus culture, but is not entirely satisfactory since it is usually desirable to at least partially sterilize the humus or other carrying material previous to inoculation with the Rhizobia culture in order to insure that the distributed organism will develop within the material to such an extent that a suitable number of organisms would be produced sufficient to inoculate the seeds to be treated. Furthermore, a large amount of metabolic products of the organisms were formed directly in the culture, which do not provide a healthy environment for the organisms, and a large percentage have a tendency to die off in a relatively short time.

A second method that has been employed for the preparation of commercial inoculants has been to develop the organism in a liquid culture and, after the development of the organism in the liquid, mix the
1085 liquid with the desired amount of carrying material such as humus or charcoal. This method again has the disadvantage that the metabolic products of

the organism and the residual sugars and protein of the culture medium are introduced directly into the commercial product as distributed, which tends to cause deterioration of the culture and the death of a large proportion of the organisms contained therein.

All of the cultures that have been successfully used in the past for the inoculation of the field leguminous plants, such as clover, alfalfa, soy beans, and the like, have contained only one species of the organism. Thus, for inoculation of soy beans, it has been the practice to use a bacterial inoculant comprising substantially a pure culture of Rhizobium japonicum in order to obtain maximum plant yield and maximum nitrogen fixation. The same is true with respect to the inoculation of the seeds of the plants belonging to the various other cross-inoculation groups which have been indicated above.

It has heretofore been considered impracticable to prepare a composite culture inoculant containing organisms which will cause nodulation on more than one of the cross-inoculation groups. This has not been done because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen fixation by the plant and the organism was inhibited or even prevented. For this reason it has been considered desirable to use a culture containing a single species of organism rather than one containing a mixture.

The use of a single species of bacteria in a culture presents a serious difficulty in the manufacture and distribution of bacterial inoculants, since it is therefore necessary to distribute a separate culture contained in a separate package for the inoculation 1086 of seeds of plants belonging to the various cross-inoculation groups. This situation is further complicated by the fact that the cultures as distributed are ordinarily usable for only a certain length of time after preparation, since the organisms in the culture tend to die out on storage of the culture on the shelves of the distributor. It has consequently become the practice among the distributors of bacterial inoculants of this character to replace cul-

tures free of charge that are unused and over about one year old.

At the present time there are on the market many so-called "pasture mixtures" of seeds which comprise a mixture of seeds including those of different species of leguminous plants. These are distributed in comparatively small quantities, and heretofore the farmer seldom takes the trouble to inoculate the mixture with the several separate bacterial cultures necessary for the seeds of each cross-inoculation group in the pasture mixture.

One object of this invention is to provide a method for the preparation of an inoculant for the seeds of leguminous plants which will contain a large number of micro-organisms and will retain their viability over a long period of time.

A further object of this invention is to provide a method for introducing a culture of bacteria into a suitable carrying material within which the inoculant is distributed.

A further object of this invention is to provide a mixed culture of Rhizobia capable of inoculating the seeds of plants belonging to several cross-inoculation groups and having the same efficiency in respect to nitrogen fixation as a culture which contains only a single species of the organism.

A further object of this invention is to provide a mixed culture the component strains of which are 1087 selected in such a manner that optimum efficiency results when the organisms are combined into a single culture.

A still further object of this invention is the provision of a bacterial culture prepared in admixture with a fine powdered inert material in a new and novel manner.

A further object of this invention is to provide an inoculant for the so-called "pasture mixture" which comprises efficient nitrogen fixing strains of different species of Rhizobia combined in one culture.

Further and additional objects of this invention will appear from the following detailed description, the drawings, and the appended claims.—

In order to prepare an inoculant in accordance with one embodiment of this invention, the desired

species of organism may be inoculated in any desired manner onto the surface of a suitable agar or jelly culture medium and allowed to grow for a period of several days. A quantity of water may then be added with thorough shaking and further growth permitted, after which time the resulting suspension may be incorporated with a suitable carrying medium such as humus, charcoal, soil, fine sand, and the like. The bacterial inoculant prepared in accordance with this general method contains an enormous number of organisms which will remain viable over a long period of time. The inoculant is easily handled and may be readily packed in cans or other containers for distribution to the farmer. If the carrying material employed is black humus or charcoal, the inoculant has a further advantage in that it may be readily determined whether or not it has been thoroughly mixed with the seeds previous to inoculation.

In order that this invention may be more readily 1088 understood, a specific example is presented in order to more definitely point out the method of preparation of the inoculant. A suitable and preferably pure culture of a bacterial species is selected. It may be obtained by direct isolation from the nodules of a leguminous plant according to methods that are well known in the art. Suitable methods are described by Fred, Baldwin and McCoy, supra, and in the Laboratory Manual of General Microbiology, by Fred and Waksman, 1928. The organism may be cultivated on a yeast extract-sucrose agar which may contain the following ingredients:

Agar	grams	15.0
Sucrose	do	10.0
Dipotassium phosphate	do	0.5
Magnesium sulfate	do	0.2
Sodium chloride	do	0.1
Calcium carbonate	do	1.0
Calcium gluconate	do	1.5
Yeast Water (pH 6.8)	cc.	50
Tap water	do	950

The yeast water infusion in the above formula may be prepared as suggested by Fred and Waksman, supra, page 16, as follows: Two kilograms of fresh

starch-free pressed yeast are added to 10 liters of tap water. The mixture is steamed for three to four hours at 95° to 100° C. with occasional stirring. The resulting mass is sterilized in an autoclave in deep layers for a period of about one hour. The sterilized material is allowed to stand for about one week, and, if undisturbed, the yeast cells will settle to the bottom and leave a clear straw-colored liquid above. The clear infusion may then be siphoned off and the reaction adjusted to pH 6.8 to 7.0. This clear infusion is the yeast water referred to in the above formula.

The ingredients of the medium are mixed together and sterilized in the usual manner. The calcium carbonate may be sterilized separately and added to the sterile medium after it has cooled down in order to prevent the decomposition of sucrose during the heating period. The medium prepared in accordance with the formula given above is a jelly on the surface of which cultures of the Rhizobia may readily develop under aerobic conditions. It may be also prepared in the liquid form by eliminating the agar.

The desired culture of the Rhizobia may be inoculated onto an agar slant of the medium and incubated at suitable temperatures for a time to permit development of the culture on the surface of the agar. This first transfer of the culture is generally made in test tubes. After a suitable period of incubation, the culture may then be transferred by a sterile needle or loop from the test tubes to an agar slant in a 200 cc. flat bottle containing about 30 cc. of slanted agar media. Here the organism is again permitted to develop for a suitable incubation period at 28° to 30° C., which in most cases will extend from a few days to over a week. The culture grown on the agar surface of the bottle may then be aseptically washed from the surface with sterile water according to recognized bacteriological technique whereby a suspension of the organisms is produced. This suspension, being quite heavy and almost opaque, may be used for the inoculation of slanted agar media contained in a plurality of still larger bottles such as one liter bottles. These bottles may contain about 125 cc. of the agar medium described above. The inoculation may be made by streaking with a needle or

aseptically spraying a water suspension of the organisms onto the surface of the agar by an atomizer or any suitable spraying means. It is desirable that aseptic conditions be maintained throughout the process until after the large bottles have been inoculated with a pure culture of the organism. Prevention of contamination up to this point is desirable, since the contaminating organism may outgrow the desired species of the Rhizobia, thus negativing the effect of the culture for seed inoculation purposes.

After the culture has developed on the surface of the agar in the large bottles for a suitable length of time (from about 2 to 10 days), tap water may be added in order to fill the bottles about half full. They are thoroughly agitated and the agar is cut or broken with a suitable metal probe, whereby the bacteria are rinsed from the surface of the agar and the agar becomes thoroughly broken up in the bottom of the bottle. It may be desirable, but it is not necessary, to employ sterile water for this purpose. The growth of the Rhizobia has taken place to such an extent that any chance contaminant will readily be suppressed by the large numbers of the Rhizobia.

After the agar has been thoroughly broken up, the large bottles are allowed to further incubate at suitable temperatures for a period of a few days. It has been found that the number of the organisms becomes very greatly increased during this subsequent period of incubation. The agar may then be separated from the suspension of organisms by passing the suspension through a suitable filter or screen. A four mesh screen has been found to be satisfactory. The bottles and agar may be washed with a small quantity of tap water. The resulting filtered suspension of bacteria is very heavy, being milk-like in appearance. This suspension may then be thoroughly incorporated with any desired amount of humus, which serves as a carrying means for the organism of the suspension. The humus and suspension mixture is allowed to stand for about 12 to 24 hours before the material is packed in order to permit complete absorption of the moisture by the humus. The resulting composition may then be packaged in moisture-proof fiber cans or other suitable containers for shipment.

The material from seventy-two one liter bottles inoculated and treated as described above is sufficient to inoculate about ninety kilograms of humus in order that the concentration of organisms in the humus will be approximately sixty million per gram. The final product may contain about 45% moisture. The water content may be adjusted by adding water in the desired proportions previous to packaging.

It will be understood, of course, that other types of media may be employed for the development of the organisms preliminary to its inoculation into the humus or other carrying material, and the proportions of the various ingredients may be varied within a wide latitude. Likewise, charcoal, fine sand, soil, kieselguhr, and other materials may also be used as the carrying medium. If humus is employed, it may be desirable to neutralize its natural acidity with hydrated lime or calcium carbonate. It is not necessary, as has been previously stated, that sterile conditions be maintained after the incubation period on the surface of the agar immediately preliminary to the addition of the excess water. Sterile conditions are not necessary after this point, since the organisms have increased to such large numbers that they will suppress any type of contaminating organism that may be introduced. Likewise, it is not necessary to sterilize the humus or other carrying material because of the tremendous number of organisms of the desired type that are introduced therein. It will be readily recognized, however, that, if desired, strict aseptic conditions may be maintained up until the time the material is packaged in the containers for shipment.

The culture prepared in accordance with this method contains a sufficient number of organisms so
1092 that 150 grams will inoculate about one bushel of small seed.

It will be understood from the above description that the number of organisms incorporated into the humus may be readily predetermined by means of a Petroff-Hausser bacterial counter or other suitable direct counting apparatus. This method, therefore, has the advantage that the number of organisms being introduced into the final inoculant may be readily

determined. The final culture is also comparatively free of excess sugar and minerals, since they are retained to a large extent by the agar in the large bottles. Consequently, additional nutrient is not introduced into the humus which may stimulate the development of contaminating organisms.

It will be understood also that the incubation period at the different stages of preparation of the culture previous to inoculating into the carrying means depends on many factors recognized by one skilled in the art. Thus, the character of the media, the temperature of incubation, and the species of Rhizobium are factors to be considered.

In order to inoculate the seeds of leguminous plants by the inoculant prepared in accordance with the above given description, the seed, within about twenty-four hours previous to the time of planting, is slightly moistened and a predetermined quantity of the inoculant is thoroughly mixed therewith in order to provide the desired concentration of organisms on the seed. The distribution of the inoculant throughout the seeds may be readily observed because of the black color of the humus with which the inoculant is prepared. The inoculant prepared in accordance with this invention may be satisfactorily used for a period of more than one year and sufficient organisms remain viable during this time in order to provide a number satisfactory for thorough inoculation.

1093 As has been pointed out above, composite cultures for inoculating the seeds of leguminous plants have not generally come into use because of the inhibitory effect that certain strains of certain species of the Rhizobia have on each other and also because no method has been developed whereby a composite culture may be produced wherein a sufficient number of each species of organism in the inoculant is insured.

In order to more clearly point out the nature and magnitude of the inhibitory effect of several species of Rhizobia, tests were carried out using strains of various organisms which were picked at random and unselected in the manner to be hereinafter described. Thus a culture of Rhizobium japonicum which was known to be an efficient culture for fixing nitrogen

when in association with the soybean plant was tested in order to determine its nitrogen fixing efficiency when inoculated onto soybean seeds in the presence of Rhizobium trifolii and Rhizobium meliloti. The tests were made by growing the appropriate plants in sterilized nitrogen-free sand according to well known methods in the art and fully set forth by Fred and Waksman, supra, and Fred, Baldwin and McCoy, supra. Three groups of soybean plants containing eight plants in each group were planted in a nitrogen-free sand containing suitable mineral salts and according to the standard methods. One group of seeds were planted sterile, being uninoculated. The seeds of the second group were inoculated with a single strain of the species of Rhizobium japonicum indicated above. The third group of seeds was inoculated with a mixture of the same strain of Rhizobium japonicum and strains of Rhizobium trifolii and Rhizobium meliloti. The seeds were permitted to germinate and the plants were grown in the sand for a period of fifty-six days. The eight plants developing from each group were then analyzed for total nitrogen by a suitable Kjeldahl procedure. The plants of the first group (uninoculated) contained a total of 79 milligrams of nitrogen. The plants of the second group (single strain) contained 246 milligrams of nitrogen. The plants of the third group (same strain mixed with strains of other species) contained only 203 milligrams of nitrogen. Consequently the plants of the second group fixed 167 milligrams of nitrogen over the control, and the plants of the third group only fixed 124 milligrams of nitrogen over the control. Thus it will be seen that this strain of Rhizobium japonicum produced 34.8% more nitrogen when employed alone than when employed in the presence of strains of other species of Rhizobia.

In the same manner a strain of Rhizobium trifolii which efficiently fixed nitrogen in the presence of the host plant alone was tested. Groups of forty plants were inoculated in this instance in the manner indicated above which is well recognized in the art. It was found that this unselected strain of Rhizobium trifolii caused about 118% more nitrogen to be fixed in the host plant when employed alone as an inocu-

lant than when in the presence of other species of the Rhizobia, such as Rhibozium japonicum and Rhizobium meliloti.

It is possible, in accordance with one embodiment of this invention, to produce an effective composite inoculant containing a plurality of species of organisms which is capable of infecting a variety of leguminous plants belonging to a plurality of cross-inoculation groups.

In general, the composite culture may be prepared by selecting strains of the desired species of the Rhizobia which will produce optimum beneficial results to the host plant and will not be adversely affected by each other. The strains, after having been selected, may be cultivated in a manner which has been described above on agar or jelly media separately, the heavy suspension of each organism being introduced directly into the humus or other carrying material in the manner that has been previously described.

In order to produce a composite inoculant containing, for example, Rhizobium meliloti, Rhizobium trifolii, and Rhizobium japonicum, which inoculant will infect the corresponding cross-inoculation groups, a number of strains of each of these organisms may be isolated from nature by any of the approved methods such as have been described in the above referred to Laboratory Manual of General Microbiology by Fred and Waksman. Each of the strains are tested separately with its own host plant in order to select out those which are most effective in respect to the development of the host plant and the quantity of nitrogen fixed thereby. These tests may be made according to standard practice by aseptically growing the plants in a nitrogen-free substrata such as sand containing mineral salts. The cultures showing the best results in association with the host plant may then be selected and the various strains of the different species may be mixed together in order to determine if the nitrogen-fixing ability of one species is adversely affected by the mere presence of another, as has been previously thought to be the case. Thus, it has been previously considered that the nitrogen-fixing ability of Rhizobium trifolii in symbiotic rela-

tionship with red clover is markedly inhibited if strains of Rhizobium meliloti, Rhizobium japonicum, or other species of the Rhizobia were present within the same inoculant. It has been found, however, that, by a suitable process of selection of strains in the manner indicated above, a composite culture may be obtained which may be used to infect a plurality of cross-inoculation groups with optimum results.

The organisms of the genus Rhizobium which are mutually noninhibitive may be designated by the term alpha. No method has been observed whereby the alpha strains of the organisms may be differentiated from the mutually inhibitive group of Rhizobia other than by making actual nitrogen fixation tests by inoculating the various host plants with the proposed mixture of the Rhizobia. A composite culture that is satisfactory for use for inoculating seeds of alfalfa, red clover, and/or soy beans (representatives of three cross-inoculation groups) contains a mixture of Rhizobium meliloti alpha, Rhizobium trifolii alpha, and Rhizobium japonicum alpha. All of the strains may be selected so as to be mutually noninhibitive when mixed and used for seed inoculation. It will, of course, be recognized that certain inhibitive strains may be employed in the mixture. In general, however, it is desirable to use the alpha strains which have been selected in the manner proposed in the above. By the alpha strains, which have been designated herein, is meant those strains of the species of Rhizobia which are mutually noninhibitive of each other as regards nitrogen fixation in the host plant. It is understood that the alpha strains of each species may vary somewhat from each other in minor physiological, morphological and/or immunological characteristics. All such variants may be included within the scope of the term alpha strain.

One criterion by which the alpha strains of the organisms may be determined is based on a statistical comparison of the total milligrams of nitrogen fixed in plants previously inoculated with a single strain unmixed with strains of other species with the total milligrams fixed in plants inoculated with the same strain mixed with strains of other species. In mak-

ing such tests the conditions for inoculation and growth of the plants should be kept uniform when testing both the mixed culture and the single strain. The method for carrying out tests for determining the amount of nitrogen fixed by leguminous plants in association with the various species of the Rhizobia are well known in the art and are given in detail by Fred and Waksman, supra, and Fred, Baldwin and McCoy, supra.

Each culture may be tested by inoculating a given number of seeds of the proper host plant with a strain of culture of bacteria to be tested either singly or mixed with other species of the Rhizobia. The seeds may then be planted in jars containing sterile sand and appropriate nitrogen-free mineral salt solutions capable of supporting plant growth. The same number of seeds are planted in each of five jars for each culture being tested, and the total number of milligrams of nitrogen fixed by the plants in each of the five jars is tested. The standard deviation of the mean amount of nitrogen fixed in each jar may then be determined by appropriate statistical methods. If the mean plus or minus the standard deviation of the nitrogen content of plants inoculated with a strain used unmixed with the strains of another species, overlaps the mean plus or minus the standard deviation of plants inoculated with the same strain used mixed with other species of Rhizobia, that strain may be considered as nonantagonistic or an alpha strain within the meaning of this invention.

For the preparation of a composite bacterial inoculant in accordance with this invention, heavy suspensions of selected cultures of Rhizobium trifolii alpha, Rhizobium meliloti alpha, and Rhizobium japonicum alpha may be prepared in the same manner as has been indicated above for the preparation of a single culture in accordance with this invention. The heavy suspensions may then be mixed and introduced into the humus in concentrations suitable to give the desired results.

In order that 90 kilograms of dry humus may be properly inoculated with the three organisms indicated above, about [19]* liters are prepared of each suspension in the manner previously indicated. The

* Matter in brackets inserted.

concentration of organisms in the suspension may be about [300]* million per cubic centimeter for Rhizobium meliloti alpha and Rhizobium trifolii alpha and about [450] million per cubic centimeter for Rhizobium japonicum alpha. The suspensions may be introduced into the humus or other carrying material separately or may be mixed before introduction. The humus, after inoculation with the three organisms (about [57] liters of composite suspension), may be passed through a suitable mechanical mixer in order to thoroughly incorporate the organisms within the humus or carrying material. The water content of the humus may be brought up to about 45% and the mass is permitted to stand for about 12 to 24 hours preliminary to packaging in order to permit a more thorough absorption of the water and bacterial cultures into the humus. This latter storage period also causes the humus to become lighter and more easily handled by the packing machines, which may be of any desired kind. One hundred seventy-five grams of this inoculant is sufficient to inoculate one bushel of red clover or alfalfa seed or two bushels of soy bean seed.

The resulting composite inoculant may be used for inoculating the seeds of leguminous plants in the same manner as hereinbefore set forth. It may be used with good results for inoculating the seeds of any of the plants included within the cross-inoculation groups represented by the bacterial species present. The particular example given has included Rhizobium trifolii alpha, since it has been generally thought that Rhizobium trifolii was particularly sensitive to other of the Rhizobia in respect to its ability to fix nitrogen within the host plant. It has been found, however, that, by suitable selecting, strains of the organism may be found from which a composite inoculant may be produced having all of the desirable qualities of a single strain culture with the further additional advantages previously indicated.

It will be understood that this invention is not to be limited to the above described method for producing a composite inoculant. It is not necessary that it be prepared as set forth in the manner described above. Any inoculant comprising a mixture of se-

* Matter in brackets inserted.

lected anti-inhibitive strains of ordinarily inhibitive organisms of the Rhizobia group is included within the spirit and scope of this invention.

In order that a more definite understanding may be had of the advantages to be derived from the use of a composite culture selected and prepared in accordance with this invention, a series of tests were made by planting red clover seeds in half gallon jars containing sterile sand supplemented with all essential minerals except nitrogen, and were inoculated as indicated below. After ten weeks of growth in the greenhouse under identical conditions, the sand was washed from the roots of the plants, the plants dried, weighed and ground and samples were analyzed by the Kjeldahl method for their nitrogen content.

Each group or series of seeds was planted in a series of five jars and the standard deviation between the jars of each culture series was determined. Each series was inoculated as follows:

1. Uninoculated control plants.
2. Plants inoculated with a non-selected strain of Rhizobium trifolii in single culture unmixed with other species.
3. Plants inoculated with a selected, non-antagonistic strain of Rhizobium trifolii alpha in single culture unmixed with other species.
4. Plants inoculated with same organism as series 2 in admixture with other species of the Rhizobia.
5. Plants inoculated with the same organism as series 3 in admixture with other species of Rhizobia.

The following table is presented which indicates the inoculant used for each series of plants, the dry weight of ten plants, the per cent of nitrogen in the dry material, the milligrams of nitrogen present per ten plants, and the milligrams of nitrogen fixed per ten plants:

Inoculant Series No.	Grams Dry Wt. Per 10 Plants	% Nitrogen	Mg. Nitrogen Per 10 Plants	Mg. Nitrogen Fixed Per 10 Plants
1	0.61	1.07	6.5 ± 0.8*	.....
2	4.18	2.92	122.0 ± 10.8*	115.5 ± 10.0*
3	4.60	2.85	131.0 ± 16.6*	124.5 ± 15.8*
4	3.53	2.29	80.8 ± 4.7*	72.3 ± 3.9*
5	4.50	2.83	126.0 ± 40.5*	119.5 ± 39.7*

* Standard deviation of the mean of a series of 5 jars.

It will be observed that the nitrogen fixing efficiency of the inoculants of series 2, 3 and 5 are high and substantially the same. When the non-selected strain, however, was mixed with other species of the Rhizobia the amount of nitrogen fixed by the host plant was seriously impaired as indicated by series 4. This example clearly shows the increase in effectiveness of a mixed culture which contains an alpha strain over that which contains a non-selected but otherwise efficiently nitrogen fixing strain. It is pointed out here that the mean plus or minus the standard deviation of the nitrogen fixed by series 4 does not fall in the same range as the mean plus or minus the standard deviation of that fixed by series 2. Series 3 and 5 do overlap in this respect and the strain of Rhizobium trifolii employed in those tests was consequently an alpha strain according to the one criterion that has been previously described.

It is to be understood that this invention is not to be limited to the selection of a single strain which is to be used in the composite culture. All of the strains may be selected in the manner indicated previous to the preparation of the inoculant. Furthermore, if desired, a plurality of strains of the same species may be introduced into the composite culture containing several species. This practice has been found to be desirable in many instances in order that there will be double insurance that an effective nitrogen fixing strain of the specific organism is present within the culture.

Referring more particularly to the drawing,

Fig. 1 is a photolithograph picture of a root system of a red clover plant having nodules A located near the crown of the root. Large nodules on the plant near the crown are indicative of good nitrogen fixation and a good strain of the organism.

Fig. 2 shows a control group of forty red clover plants which have grown in a nitrogen-free sand culture for ten weeks with no Rhizobia present. This group had a wet weight of 20.4 grams and total nitrogen content of 27 milligrams derived from the original seeds. No nitrogen was fixed.

Fig. 3 shows the forty-one plants grown under identical conditions as those shown in Fig. 2 but which

were inoculated with a humus inoculant containing only bacteria of the species Rhizobium trifolii alpha prepared in accordance with this invention. The wet weight of this group of plants was 156.7 grams and had a total nitrogen content of 645 milligrams of nitrogen. Thus 618 milligrams of nitrogen were fixed.

Fig. 4 shows a group of forty plants the same as Fig. 3 which were inoculated with a composite inoculant prepared in accordance with one embodiment of this invention containing strains of the species Rhizobium trifolii alpha, Rhizobium meliloti alpha, and Rhizobium japonicum alpha. It will be noted that the red clover plants inoculated with the composite inoculant containing the alpha strains are fully developed to the same extent as the one inoculated with the pure culture and no inhibition is observed in the plant development because of the presence of the other organisms. The wet weight of this group was 159.0 grams and had a total nitrogen content of 662 milligrams. Thus 635 milligrams of nitrogen were fixed.

It will be observed that the organisms of the composite culture described in the foregoing example may be introduced into the culture in any desired numbers and the relative proportion may be fairly accurately predetermined by means of direct bacterial count. The cultures are not mixed until immediately preliminary to their introduction into the carrying material and after they have developed to substantially their maximum numbers. Consequently, there is no danger of one species overgrowing the other during the process of incubation.

The invention is not to be limited in any way to the 1103 number of organisms contained within the inoculant or the number necessary for inoculating a predetermined quantity of seed. In practice, and as shown herein, a large excess of bacteria is generally introduced into the carrying material in order to insure that all of the seeds are thoroughly inoculated with a sufficient number of organisms to produce the desired results.

While several particular embodiments of this invention are shown above, it will be understood, of course,

that the invention is not to be limited thereto since many modifications may be made, and it is contem-
10 plated, therefore [by the appended claims]* to cover any such modifications as fall within the true spirit and scope of this invention as defined by the appended claims.

I claim:

1104 1. A method for producing an inoculant for leguminous plants of the character described which comprises inoculating the surface of a jelly nutrient medium with a culture of bacteria of the genus Rhizobium, incubating the culture on the jelly to permit growth of the bacteria, adding water to the jelly culture, further incubating said culture and incorporating the resulting water suspension of bacteria with an inert, finely comminuted base material.

2. A method for producing an inoculant for leguminous plants of the character described which comprises aseptically spraying the surface of an agar nutrient medium with a culture of bacterium of the genus Rhizobium, incubating the culture on the agar to permit growth of the bacteria, adding water to the agar culture, breaking up the agar mass in the water, further incubating said culture and incorporating the resulting water suspension of bacteria with powdered humus.

3. A method for producing an inoculant for leguminous plants containing inappreciable amounts of nutrient material which comprises growing bacteria of the genus Rhizobium on the surface of a solid nutrient medium, washing said bacteria from the surface with water, incubating further to permit further growth in the wash water in the presence of said solid medium, separating the solid medium from the bacterial suspension, and incorporating the suspension with neutralized humus.

1105 4. In a process for producing an inoculant for leguminous plants of the character described, the improvement which comprises growing bacteria of the genus Rhizobium on the surface of a solid nutrient medium, washing said bacteria from the surface with water, and incubating further to permit further growth in the wash water in the presence of said solid medium.

* Matter in brackets added.

1. 5 An inoculant for leguminous plants comprising a plurality of selected cultures of different species of bacteria of the genus Rhizobium, one of said cultures being Rhizobium trifolii alpha, said cultures being substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

2. 6 A bacterial inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium comprising Rhizobium trifolii alpha, Rhizobium meliloti alpha, and Rhizobium japonicum alpha.

3. 7 An inoculant for leguminous plants comprising humus and a mixture of species of bacteria of the genus Rhizobium, said mixture containing a strain of Rhizobium trifolii alpha.

. 8. An inoculant for leguminous plants comprising humus and a mixture of species of bacteria of the genus Rhizobium, said mixture containing a strain of Rhizobium trifolii alpha, said strain of Rhizobium trifolii alpha being characterized by its ability to fix nitrogen in the host plant in the presence of other species of Rhizobia with substantially the same efficiency as when used alone in said inoculant.

1106 9. An inoculant for leguminous plants comprising a plurality of selected strains of different species of bacteria of the genus Rhizobium, comprising Rhizobium trifolii alpha, Rhizobium meliloti alpha, and Rhizobium japonicum alpha in moist powdered humus.

10. An inoculant for leguminous plants comprising a moist powder base, bacteria of the species Rhizobium trifolii, and bacteria of another species of the genus Rhizobium.

11. An inoculant for leguminous plants comprising a moist powder base, bacteria of the species Rhizobium trifolii, and bacteria of another species of the genus Rhizobium, the numbers of bacteria of each species within the inoculant being of substantially the same order of magnitude.

4. 12 An inoculant for leguminous plants comprising a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus Rhizobium, said strains being unaffected by each

other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

13. An inoculant for leguminous plants comprising a moist powder base and a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus Rhizobium, said strains being unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

1107 5. 14 An inoculant for leguminous plants comprissing a plurality of strains of different species of bacteria of the genus Rhizobium, at least one of which strains is unaffected by the others in respect to its ability to fix nitrogen in the leguminous plant for which it is specific.

6. 15 An inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium, the bacteria of each strain being present in numbers of substantially the same order of magnitude, said strains being substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

10. 16 A method for producing a composite inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium, which comprises cultivating each strain separately in suitable culture medium to produce a substantial quantity of living organisms and thereafter mixing the organisms with a moist powder base containing a substantially negligible concentration of bacterial nutrients whereby the desired inoculant is formed.

17. A method for producing a composite inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium, which comprises cultivating each strain separately on a solid culture medium, washing the bacteria from said medium with water, incubating further in said water in the presence of said medium, and mixing the resulting bacterial suspensions whereby the desired inoculant is formed.

1108 18. A method for producing a composite inoculant for leguminous plants comprising a plurality of

strains of different species of bacteria of the genus Rhizobium, which comprises cultivating each strain separately on a solid culture medium, washing the bacteria from said medium with water, incubating further in said water in the presence of said medium, mixing the resulting bacterial suspensions, and incorporating the mixture into a dry inert powder base.

19. A method for producing a composite inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium, which comprises cultivating each strain separately on a solid culture medium, washing the bacteria from said medium with water, incubating further in said water in the presence of said medium, mixing the resulting bacterial suspensions, and incorporating the mixture into neutralized humus.

20. All novel features in their broadest aspect not otherwise specifically claimed which are disclosed, or are inherent, in the accompanying specification and/or drawings.

1109 In witness whereof, I have hereunto subscribed my name.

Varley Sherman Bond.
(Inventor sign here, including first name in full)

OATH.

State of Illinois }
County of Adams } ss.

Varley Sherman Bond the above named petitioner, being sworn, deposes and says that he is a citizen of the United States and resident of Quincy in the County of Adams and State of Illinois; that he verily believes himself to be the original, first and sole inventor of the improvement in Bacterial Inoculant for Leguminous Plants described and claimed in the annexed specification; that he does not know and does not believe that the same was ever known or used before his invention or discovery thereof, or patented or described in any printed publication in any country before his invention or discovery thereof, or more than two years prior to this application, or in public use or on sale in the United

States for more than two years prior to this application; that said invention has not been patented in any country foreign to the United States on an application filed by him or his legal representatives or assigns more than twelve months before this application; and that no application for patent on said improvement has been filed by him or his representatives or assigns in any country foreign to the United States.

VARLEY SHERMAN BOND

(Inventor sign here, including first name in full, and also on line indicated at top of this page)

Subscribed and sworn to before me this 20th day of August, 1938.

RICHARD C. MURRAY
Notary Public.

My commission expires 8/27/41

(Notary must place seal here)
(SEAL)

226565

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9-07

Fig 1

Fig 2

Fig 3

Fig 4

Inventor
Varley Sherman Bond
By Amos Thurston Olin & Hopkinson
Attys

59 — 30 — 71-7

1111 DEPARTMENT OF COMMERCE
United States Patent Office
Washington

Please find below a communication from the EXAMINER in charge of this application.

CONWAY P. COE,
Commissioner of Patents,

Ames, Thiess, Olson &
Mecklenburger
77 West Washington St.
Chicago, Illinois

Applicant: Varley S. Bond
Ser. No. 226,565
Filed Aug. 24, 1938
For Bacterial Inoculant
for Leguminous Plants.

Mailed Nov. 9-1938

This application has been examined.
References made of record:

Hendrickson	2,098,918	Nov. 9, 1937	195-54
Bottomley	982,569	Jan. 24, 1911	71-7
Coates	899,155	Sept. 22, 1908	71-7
Stoltz	1,442,239	Jan. 16, 1923	71-7
Earp-Thomas	1,099,121	June 2, 1914	71-6

Coates, Stoltz, and Earp-Thomas teach the suspension of prepared bacteria cultures in humus, phosphate rock, calcium carbonate, soils and prepared fertilizers in general.

Bottomley shows the combination of bacteria culture with bacterial food, and a carrier like humus, etc.

Hendrickson teaches the methods of preparing the cultures of the species of Rhizobium which are effective for the various types of plants.

The applicant has admitted that his alleged invention lies in the use of cultures of a new strain or species of bacteria which do not show the inhibiting effect of one species upon another. Claims 1-4, 10, 11 and 16-19 do not appear to be drawn to the alleged invention and are therefore rejected.

Claims 1-3, 18 and 19 are rejected on Hendrickson in combination with any of Coates, Stoltz or Earp-Thomas.

Claims 4, 10, 11, 16 and 17 are rejected on Hendrickson.

In claims 5-9, drawn to the alleged invention, the new strain of bacteria is referred to as Rhizobium trifolii alpha which name has been applied by the applicant. This is indefinite and confusing as the name has not been used before and the applicant does not known definitely whether a new organism has been isolated. 1112 A better designation of the bacteria culture would be to identify it by its properties.

Claims 5-9 are therefore rejected as indefinite and confusing.

Claims 12-15 appear to be allowable.

P. W. SHEPARD
Examiner

1113

IN THE UNITED STATES PATENT OFFICE

(Stamps) Mail Division, April 29, 1939.

U. S. Patent Office.

U. S. Patent Office May 1, 1939. Division 59.

Application of
Varley Sherman Bond
Serial No. 226,565
Filed August 24, 1938
For Bacterial Inoculant
In Leguminous Plants

} Division 59, Room 4709.

Honorable Commissioner of Patents
Washington, D. C.

Sir:

In response to the Office Action dated November 9, 1938, paper No. 3, please amend the above-identified application as follows:

In the specification, page 18, line 9, cancel "23" and insert therefor 19

line 12, cancel "500" and insert therefor 300

line 13, cancel "750" and insert therefor 450

line 17, cancel "70" and insert therefor 57

Claim 3, line 2, cancel "a small amount" and insert therefor inappreciable amounts

Withdraw claim 20.

Add the following claims:

21. A bacterial inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium including a strain of Rhizobium trifolii, said strain of Rhizobium trifolii being unaffected by the others in respect to its ability to fix nitrogen in the leguminous plant for which it is specific.

1114 22. An inoculant for leguminous plants comprising bacteria selected from a plurality of strains of different species of the genus Rhizobium, at least one of

said strains being identifiable as Rhizobium trifolii which is unaffected by the other strains present in the inoculant in respect to its ability to fix nitrogen in the leguminous plant for which it is specific.

7. 23 A bacterial inoculant for leguminous plants comprising a moist powder base and a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus Rhizobium including Rhizobium trifolii, the bacteria of each strain being present in numbers of substantially the same order of magnitude, said strains being substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

8. 24 A bacterial inoculant for leguminous plants comprising a moist powder base containing a substantially negligible concentration of bacterial nutrients and a plurality of strains of different species of bacteria of the genus Rhizobium, at least one of which strains is unaffected by the others in respect to its ability to fix nitrogen in the leguminous plant for which it is specific.

9. 25 A method for producing a bacterial inoculant for leguminous plants which comprises separately selecting strains of different species of the genus Rhizobium, separately cultivating said strains in nutrient media, and mixing the resulting cultures whereby a composite inoculant is produced, said strains originally being selected so that they are substantially non-inhibitive and non-antagonistic to each other in respect to their ability to fix nitrogen in the host plant for which they are specific.

1115 11. 26 A method for producing a composite bacterial inoculant for leguminous plants which comprises separately cultivating a plurality of strains of bacteria selected from different species of the genus Rhizobium and subsequently mixing the resulting cultures containing large numbers of bacteria whereby a mixture of strains of the bacteria is obtained, the bacteria of each strain being present in numbers of substantially the same order of magnitude.

Respectfully,

VARLEY SHERMAN BOND

By Ames, Thiess, Olson & Mecklenburger

Attorneys.

REMARKS:

The specification has been amended in order to describe a preferred method for incorporating the bacteria with the dry humus in desired numbers in accordance with this invention. The amendment is only directed to specific amounts of bacterial culture that may be added to the humus to produce a commercially desirable inoculant.

Claim 20 has been withdrawn from the case.

The Examiner has indicated in the last Office Action that claims 12 to 15, inclusive, will be allowed.

Claims 5 to 9, inclusive, have been rejected on the basis of the terminology employed in relation to the strains of bacteria contemplated by the applicant in his present invention.

Claims 1 to 4, inclusive, 10, 11, and 16 to 20, inclusive, were rejected on certain references that were cited by the Examiner, and also on the basis that they do not appear to be drawn to the applicant's invention.

1116 *The Invention and the Prior Art*

Before discussing the claims specifically it is believed desirable to make a few remarks about the present invention and its relation to the prior art which has been cited by the Examiner. The applicant has broadly discovered a new method for producing bacterial inoculants for leguminous plants and a composite bacterial inoculant which has not been previously described in the art. The specification discloses a number of closely interrelated and inseparable inventions for which the applicant wishes to obtain patent protection. Claims have been drawn both to a process and product in an attempt to suitably cover all the aspects of the invention. The invention includes a method of cultivating bacteria of the genus Rhizobia in such a manner that the inoculant produced therefrom will contain a large number of organisms in the absence of appreciable amounts of nutrient material derivable from the medium upon which the organism was grown. The applicant has also discovered that a composite inoculant may be produced by combining selected strains of various species of the Rhizobia in a particular manner described in the specification. It is stated in the specification on pages 6 and 7 that these are among the objects of the present inven-

tion, and the Examiner's contention that certain of the claims do not appear to be drawn to the invention as defined by the applicant is clearly in error.

The patent principally relied on by the Examiner in the rejection of the process claims and claims 10 and 11 appears to be patent No. 2,098,918 to Hendrickson. The Hendrickson patent teaches nothing more than the separate growth of Rhizobia on the surface of silica gel medium. This process, however, has nothing whatsoever to do with the process contemplated by the applicant. Claims 1 to 4 and 17 to 19 are all directed to a process for growing bacteria on a solid surface which includes the step of washing the organisms from the surface of the medium and permitting them to incubate further, directly in the wash water that is employed. It has 1117 been found that by this process the number of bacteria that can be obtained from a single jelly slant is very greatly increased. The result is entirely unexpected since a bacteriologist would ordinarily presume that all of the nutrient material had been exhausted while the organism was growing on the surface of the slant and consequently no more development would take place. The applicant has described a means for producing greater numbers of organisms from the same culture medium by the expedient of washing the organisms from the surface and permitting them to develop further directly in the wash water in contact with the solid medium. Hendrickson does not disclose this step nor does any of the art which has been cited by the Examiner. In so much as claims 1 to 4, inclusive, and 17 to 19, inclusive, are drawn to a method which includes this step, it is believed that they are clearly allowable over the art. Claims 10 and 11 were also rejected on the Hendrickson patent. These claims are directed to a composite culture containing the species Rhizobium trifolii. Hendrickson does not even suggest a composite culture and he certainly does not describe one which comprises Rhizobium trifolii admixed with another species of Rhizobium.

Coates in patent No. 899,155 merely describes a composition of matter containing symbiotic nitrogen fixing bacteria admixed with meal and added to fertilizer. Only

a single species of the organism is contemplated and there is no suggestion of either a composite culture or a method such as disclosed by applicant.

The Stolz patent No. 1,442,239 discloses a composition of matter containing the nitrifying bacteria incorporated with a humus, lime and phosphate powder base. The Examiner's attention is directed to the fact that the nitrifying bacteria are not even closely related to the symbiotic nitrogen fixing bacteria which are contemplated by the applicant in the present invention. The nitrifying bacteria function in the soil to transform 1118 inorganic ammonia compounds to nitrites (Nitrosomonas) and nitrites to nitrates (Nitrobacter). The species of Rhizobia contemplated by the applicant, however, act only in symbiotic relationship with the leguminous plants to convert atmospheric nitrogen directly to organic nitrogen within the plant. While Stolz may show the use of humus as a base for a bacterial culture, the applicant does not attempt to claim the use of humus as such per se. He has discovered that the humus is a desirable carrier for the new inoculant prepared in accordance with his invention. Consequently it is not believed that Stolz should be considered anticipatory of any of the claims in the present application, either alone or in combination with any of the other art of record.

Earp-Thomas in patent No. 1,099,121 likewise incorporates bacteria with a soil or humus base. Again, however, it is not believed that this reference is applicable since the novelty of the claims of the present case does not depend entirely on the fact that the organism is mixed with humus or other moist powder base. Applicant's method of cultivating the organisms is not anticipated by this patent.

Patent No. 982,569 to Bottomley describes a process for producing a culture beneficial to soil which comprises a mixture of Pseudomonas radicicola mixed with the culture of Azotobacter. The Pseudomonas described by Bottomley is apparently actually one of the Rhizobia since he states that it is capable of fixing nitrogen while in symbiotic relationship with certain of the leguminous plants. Azotobacter, however, are a well defined bacterial genus which belong to that group of bacteria which fix small quantities of nitrogen in the soil directly, no

symbiosis between it and a higher plant being required. Consequently, Bottomley appears to describe merely a mixture of cultures, one of which belongs to the genus Rhizobia and the other of which belongs to the genus Azotobacter which is a genus entirely unrelated to the Rhizobia. (See Bergey's Manual of Determinative Bacteriology, Williams and Wilkins, 3rd Edition, 1930.) Furthermore, Bottomley contemplates that the culture which he produces is to be inoculated directly into soil upon which non-leguminous plants are to be grown. The effectiveness of this treatment is open to considerable doubt since it is well recognized that the non-leguminous plants such as the grains and cereals are incapable of fixing atmospheric nitrogen either alone or in symbiotic relationship with any bacteria.

1119

Claims Directed to the "Alpha" Strains

The Examiner has rejected claims 5 to 9, inclusive, on the ground of indefiniteness because the applicant has designated the strains of bacteria selected as the alpha strains of the various species of the Rhizobia, such as Rhizobium trifolii alpha, Rhizobia meliloti alpha, and Rhizobium japonicum alpha. It is submitted that the rejection of these claims to the product on this ground is clearly unwarranted. The applicant has gone to great length in the specification to define those particular strains of each species which are used in preparing the composite inoculant described in the present application. It has not been heretofore recognized that certain strains of the Rhizobia are non-inhibitive and non-antagonistic to each other in respect to their ability to fix nitrogen in symbiotic relationship with leguminous plants. The applicant has discovered that, by suitable selection, strains of the various species can be obtained which are mutually non-inhibitive. These particular strains he has chosen to designate by the term "alpha." This practice is clearly not objectionable, since the applicant has the right to employ any terms that he may desire in the claims, so long as they are adequately defined in the specification, whereby one skilled in the art could practice the invention described by the applicant.

The Examiner's attention is directed to the group of patents tha are being continually issued by the 1120 Patent Office from Division 63 relative to fermentation processes carried out by bacteria of the genus Clostridium for the production of butyl alcohol from carbohydrate mashes. With regard to the inventor's right to select and use coined names which depart from the system of names previously used, the Examiner's attention is directed to the following patents:

2,123,078—P-Bacillus
2,096,377—Clostridium saccharo-butyl-isopropyl-acetonicum
2,089,562—Clostridium inverto-acetobutylicum
2,017,572—Clostridium viscifaciens
2,085,428—Clostridium viscifaciens
1,933,682—Bacillus technicus

All of these names have been applied to butyl alcohol fermenting organisms.

The Examiner's attention is further directed to certain recent patents which have issued from the fermentation division which contain claims that are directed to particular strains of a species of an organism similar to the designation employed by the applicant in the present invention. Thus, patent No. 2,063,450 contains claims directed to an alpha strain of Bacillus acetoethylicum. Patent No. 2,016,112 is directed to a gamma strain of Clostridium saccharobutylicum. Patent No. 2,050,219 contains separate claims which are directed to the beta and gamma strains of Clostridium saccharo-acetobutylicum. The organisms described in the claims of these last three mentioned patents are strains of recognized species which were artificially set up by the patentees in the specification in order that they may be more definitely recognized.

The applicant has attempted to use the same approach in the present application in defining the alpha strains of the organisms employed in claims 5 to 9, inclusive. In the specification on pages 16, 17, 19, 20, and 21, a

definite method is pointed out for distinguishing the
1121 alpha strains from other strains of organisms
of the same species. It is, therefore, believed that the term "alpha" as used in the claims is definite and clear in view of the full description in the specification.

Discussion of Individual Claims

In view of the remarks that have been previously made, the Examiner is requested to reconsider his rejection of the originally filed claims and to consider the newly added claims 21 to 26, inclusive.

Claims 1 through 4 are directed to a process for producing an inoculant which comprises inoculating a bacteria of the species Rhizobia on a solid surface, permitting them to develop thereon, adding water to the solid culture, further permitting development of bacteria in the water, and preparing the inoculant from the resulting bacterial suspension. As pointed out, the steps of this process are nowhere suggested in any of the art that has been cited by the Examiner. While it may be true that the development of bacteria on jelly surfaces is old and the incorporation of bacteria into a powder base such as humus is also old, the step of incorporating wash water to the surface of a fully developed jelly culture and further incubating the same, whereby a great increase in numbers of the organisms is obtained, is believed to be new with the applicant. It is believed that he is entitled to patent protection therefor.

Claim 3 has been amended in order to more definitely point out that the neutralized humus with which the culture is incorporated contains only inappreciable amounts of bacterial nutrient material which is desirable for a culture of this type. Appreciable amounts of nutrient do not diffuse from the agar into the wash water while the culture is being incubated in the presence of the water. Consequently, large amounts of nutrient are
not introduced into the humus, as is the case when
1122 cultures that have been entirely developed in liquid
media are introduced therein.

Claims 5 to 9, inclusive, are all directed to a composite inoculant containing a plurality of species of the

Rhizobia, at least one of which is an alpha strain. In view of the full disclosure made in the specification, wherein the alpha strain was defined, and the remarks that have been made above, it is believed that these claims are clearly allowable along with allowed claims 12 to 15, inclusive. The applicant has the right to claim his invention in several different ways if he so desires, so long as the number of claims is not unduly multiplied.

Claims 10 and 11 are directed to an inoculant containing a plurality of species of the Rhizobia including Rhizobium trifolii. A culture of this type is particularly desirable for inoculating the so-called "pasture mixture" of seeds, said mixture usually containing red clover seeds which develop to produce the host plant for which Rhizobium trifolii is specific.

The Examiner has indicated that claims 12 to 15 are allowable.

Claim 16 relates to a method for producing a composite inoculant which comprises separately cultivating a plurality of strains of different species of the bacteria of the genus Rhizobium and subsequently mixing the cultures thus produced in order to produce the desired composite inoculant. The process defined by this claim has not been suggested in any of the art of record. It has been customary in the past, when producing mixed bacterial cultures, to grow the mixture together in one culture. This process is unsatisfactory, howe er, since one species of the organism has a tendency to outgrow the other. Consequently, the resulting culture may contain a relatively large amount of organisms of one species but a comparatively small amount of organisms of another. Such a culture is unsatisfactory for inoculating the seeds of a pluraiity of types of legume cultures which are capable of being infected with the various species
1123 of Rhizobia carried within the inoculant. The process of separately developing the organisms permits them to be introduced separately and in full strength into a powder base material if such is actually employed for the production of the inoculant. The full number introduced into the base may be predetermined.

Claims 17, 18, and 19 are similar to claims 1 through 4, except that they are directed to the preparation of

a composite bacterial culture by separately growing the organisms on a solid culture media, washing the organisms from said media, and permitting them to further develop in the wash water, subsequently mixing the separate cultures so produced to produce the desired inoculant. For the reasons stated above, these claims are also deemed to be clearly allowable.

Claim 20 has been withdrawn from the case.

Newly added claims 21 to 23, inclusive, are directed along the lines of the allowed claims 12 to 15. They are specifically directed to a bacterial inoculant containing Rhizobium trifolii. These claims have been directed specifically to this organism, since it has been considered in the past that it is this organism that was particularly susceptible to the antagonistic action of other species of the Rhizobia when it infects the roots of the host plant for which it is specific. The sensitivity of this organism is particularly pointed out on pages 13 and 14 of the specification. The marked improved results obtained by the strain selected in accordance with the applicant's invention has been pointed out.

Claim 24 is similar to allowed claims 12 to 15, but is more specifically directed to an inoculant which contains substantially negligible quantities of bacterial nutrient in the powdered humus. Such a product is readily obtainable by the method described in process claims 17 to 19. The low nutrient content of the composite culture prevents substantial development of either organism within the material and the numbers of each strain remain in a more or less dormant, although viable, state for long periods of time.

1124

Claim 25 is directed to a method for producing a composite culture which comprises first selecting strains of organisms which are substantially non-inhibitive, separately cultivating them and mixing the cultures to form the desired composite inoculant. The method described in this claim has been clearly described in the specification and is, therefore, believed to be allowable.

Claim 26 is similar to claims 16 and 25, but is directed to a means for producing a bacterial inoculant having large numbers of bacteria of a plurality of strains, the bacteria of each strain being present in numbers of substantially the same order of magnitude.

The applicant has spent considerable time and effort in the preparation of this amendment in an attempt to overcome the Examiner's rejection of claims 1 to 11 and 16 to 19. In view of the remarks that have been made above and in the absence of further prior art, it is believed that they, along with the newly added claims 21 to 26, are clearly allowable. Such action is respectfully requested.

Respectfully submitted,
Ames, Thiess, Olson & Mecklenburger
Attorneys for Applicant

Chicago, Illinois
April 27, 1939

Div. 59 Room 4709 DJH:tis Paper No. 5

1125 DEPARTMENT OF COMMERCE
United States Patent Office
Washington

Please find below a communication from the EXAMINER in charge of this application.

CONWAY P. COE,
Commissioner of Patents,

Ames, Thiess, Olson &
Mecklenburger
77 West Washington Street
Chicago, Ill.

Applicant: Varley Sherman Bond
Ser. No. 226,565
Filed Aug. 24, 1938
For Bacterial Inoculant
for Leguminous Plants
(Stamp) Mailed Sep. 12, 1939

Responsive to amendment filed Apr. 29, 1939.

Additional reference:

Buchanan—"Bacteriology" pp. 120-123, MacMillan Co. (1938) Copy in Div. 63.

The Draftsman has reported that the drawing is informal and not acceptable because the figures are blurred when prints are made. A new drawing with figures on a larger scale will be required.

It appears to be the practice in the division handling the bacteriology arts to permit the claiming of a new

bacteria by a name given by the inventor. The objection to this terminology will not be pressed further but attention of the applicant is called to claims 12-15 and 21-26 which are drawn to same subject matter and are of substantially the same scope as claims 5-9. Applicant will not be permitted to claim the subject matter both by the arbitrarily assigned name and by a description of its properties. Claims 5 and 21 are substantially identical and cancellation of one is required.

The Buchanan reference teaches the elementary principles of bacteria culture. Both liquid and solid culture mediums are widely used each having its peculiar adaptations. In the use of solid mediums the bacteria growth occurs on the surface while in liquid mediums the growth may occur on the surface or under the surface depending on whether or not air is essential to the growth of the particular bacteria. On page 1, column 1126 2, lines 8 et seq. of Hendrickson the procedure is described for removing bacteria from a culture by washing with water to obtain a suspension of the organism in the wash water. Hendrickson uses the water suspension to moisten seeds but in view of Earp-Thomas and Coates there is no invention in using the same water suspension to moisten soil, fertilizers or other carrier agents. It appears obvious that the culture medium may be reused for further incubation. The breaking up of the agar mass to provide additional surface for bacterial growth is a logical procedure which follows from the applicant's desired results which are to produce as many bacteria as possible. No invention is seen in claims 1-4 over Hendrickson and Buchanan in view of any of Stoltz, Earp-Thomas or Coates. Further, there is no relationship between the general cultural methods of claims 1-4 and 16-19 which are obviously adapted to the culture of any bacteria and the new non-inhibitive strain of bacteria which is the subject matter of claims 5-9, 12-15 and 21-25.

Claims 10 and 11 are rejected on the combination of Hendrickson with any of Earp-Thomas, Stoltz and Coates who teach the use of powdered carriers. Hendrickson shows that there are several species of Rhizobium and that each species of legume has a specie of Rhizobium with which it grows best. It appears to be customary to inoculate soils or fertilizers with that specie of bac-

teria suited to crop to be grown. When a plurality of crops are to be grown it is obvious to mix a plurality of bacteria species.

The applicant has acknowledged as part of the prior art (page 5 of specification) that the presence of more than one species of bacteria in a culture will cause the inhibition of all. It is submitted that where a product is required containing a plurality of species of which 1127 may not be cultivated together because of the inhibitory effect, it is elementary to grow them separately and combine them in the final mixture. A very clear teaching of this is found in Earp-Thomas (page 2, line 55 et seq.) He states that soils may be inoculated with a single or a variety of species of bacteria which may be grown in a single or separate cultures. Obviously if the species are known to be mutually inhibitory separate cultures will be used. Thus claims 10, 11, 16 and 26, are unpatentable over either Hendrickson or Earp-Thomas.

Claims 17, 18 and 19 are rejected as unpatentable over either Hendrickson or Earp-Thomas who show the use of plurality of bacterial strains with the conventional culture procedure as taught by Hendrickson and Buchanan and with the inert carrier as used by Coates, Earp-Thomas and Stoltz.

Claims 5-9, 12-15 and 21-25 are allowable.

Attention of applicant is called to the minute distinctions which exist between certain of the claims indicated as allowable. Examiner believes that the invention is of such nature that it can be covered completely by half of number of claims now in the case. Applicant will, therefore, be required to limit his claims under the doctrine of Ex parte Schwartz 1935 C. D. 23 and to differentiate clearly each from the other.

Claims 1-4, 10, 11, 16-19 and 22 and 26 are rejected.

P. W. SHEPARD
Examiner.

1128 IN THE UNITED STATES PATENT OFFICE

U. S. Patent Office
Feb. 9, 1940
Division 59

Div. 59 Room 4709
Application of
Varley Sherman Bond
Filed Aug. 24, 1938
Serial No. 226,565

Hon. Commissioner of Patents:
Washington, D. C.

Sir:

The undersigned, attorneys of record in the above-entitled case, hereby appoint Thiess, Olson & Mecklenburger, a firm composed of J. Bernhard Thiess, Arthur A. Olson, A. F. Mecklenburger, Willard L. Pollard, George W. Hansen, A. Arnold Brand, Thorley von Holst, Bertram Wm. Coltman, Albert H. Pendleton, Carl Marx, Arthur B. Seibold, Jr., and Sidney Neuman, of 77 West Washington Street, Chicago, County of Cook, and State of Illinois, substitute attorneys in their place and stead, with power of substitution and revocation, to prosecute the above application, to make alterations and amendments therein, to receive the patent, and to transact all business in the Patent Office connected therewith.

Chicago, County of Cook, State of Illinois, this 1st day of February, 1940.

Ames, Thiess, Olson & Mecklenburger
Attorneys.

1129 IN THE UNITED STATES PATENT OFFICE

U. S. Patent Office
Mar. 12, 1940
Division 59

Application of
Varley Sherman Bond
Serial No. 226,565
Filed August 24, 1938
For Bacterial Inoculant
for Leguminous Plants

Division 59, Room 4709.

HON. COMMISSIONER OF PATENTS
Washington, D. C.

Sir:

In response to the Office Action dated September 12, 1939, Paper No. 5, please amend the above-identified application as follows:

In the specification, page 8, line 23, cancel "is" (first occurrence) and insert therefor are

Page 23, line 10, before "to" insert by the appended claims

line 12, cancel "as defined by the appended claims"

In the claims, withdraw claims 1, 2, 3, 4, 8, 9, 10, 11, 13, 17, 18, 19, 21, and 22.

Claim 16, line 6, after "organisms" insert with a moist powder base containing a substantially negligible concentration of bacterial nutrients

Claim 26, line 6, cancel "whereby a mixture of strains of the bacteria" and substitute therefor with a moist powder base containing a substantially negligible concentration of bacterial nutrients whereby the desired inoculant

1130 Add the following claims:

12. 27 A method for producing a composite inoculant for leguminous plants which comprises separately cultivating a plurality of species of bacteria of the genus Rhizobium in separate cultures and mixing with a powder base predetermined numbers of the bacteria of each cul-

ture to produce the desired inoculant, said powder base being substantially free of added bacterial nutrients.

13. 28 A bacterial inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium in numbers of substantially the same order of magnitude incorporated with a powder base, the mixture being substantially free of bacterial nutrients whereby growth of the bacteria therein is inhibited.

14. 29 The inoculant as defined in claim 28 [13]* wherein one of said species is Rhizobium trifolii.

Respectfully,
VARLEY SHERMAN BOND
By Thiess, Olson & Mecklenburger
Attorneys.

REMARKS:

Minor changes have been made in the specification. Claims 1 through 4, 8 through 11, 13, 17 through 19, 21, and 22 have been withdrawn from the case. Claims 16 and 26 have been amended. Claims 27, 28, and 29 have been added. In view of the amendments that have been made, the Examiner is respectfully requested to consider the claims as they now stand in the case.

The Examiner has indicated in the last Office Action that a new drawing with figures on a larger scale is required. Accordingly, a new sheet of drawings is enclosed herewith which shows enlarged reproductions of the same plants and root system that were shown in the drawings originally filed in the case. It is believed that these drawings are in such condition as to overcome the objection made by the Official Draftsman. If, however, the new drawings are unsatisfactory, the applicant will furnish the prints from which they were made so that the Official Draftsman may make drawings to his own satisfaction in accordance with Rule 55 of the Rules of Practice.

The Examiner has objected that there is no relationship between the cultural method such as recited in claim 16 and the new noninhibitive strain of bacteria which is claimed in the allowed claims in the case. It is submitted that the Examiner is laboring under a misapprehension as to the true nature of the applicant's present invention. While it is true that the applicant has disclosed and claimed a new type of noninhibitive strain of the several

* Matter in brackets added

Rhizobia which may be employed in the preparation of a composite culture, he has also disclosed a new and satisfactory method for producing composite cultures in general which have not been disclosed or even remotely suggested in any of the references of record, and it is believed that he is entitled to claims directed to this new process. It is clearly pointed out in the specification as filed that certain objects of the invention are to provide a method or methods for introducing a bacterial culture into a suitable carrying material whereby a bacterial inoculant is produced which will retain its viability and effectiveness over long periods of time. Thus, a process has been described for incorporating a plurality of strains of organisms into a single culture in such a manner that each of the strains so incorporated will be present in predetermined numbers and will maintain its viability throughout the normal commercially useful period of the culture. One means for obtaining this result has been to incorporate into the composite culture strains of mutually noninhibitive bacteria. This is but one of the important factors. In a composite culture such as proposed by the applicant it is important that each species be grown separately before mixing, that each species be present in the mixed culture in a definite predetermined proportion, and that the base be substantially free from bacterial nutrients. This is necessary so that the culture may be effective in inoculating the seeds of any of the plants of the several cross inoculation groups for which the inoculant was originally prepared.

1132

Earp-Thomas has indicated that a mixed culture may be prepared by inoculating several organisms into a soil base with which have been incorporated substantial quantities of carbohydrates or other bacterial nutrients (see page 2, lines 5-8). Earp-Thomas contemplates that only relatively small numbers of each species be inoculated into the nutrient-containing moist base and he then relies on subsequent growth of each culture therein to produce the desired composite inoculant. This process is not satisfactory because the more rapidly growing strains outgrow those strains which develop more slowly. Thus, when the composite culture is prepared in accordance with the disclosure in Earp-Thomas, certain strains, such as a culture

of Rhibozium trifolii, will develop rapidly in the nutrient-containing moist base, due to their rapid growth characteristics, while a slower growing strain, such as Rhizobium japonicum, is suppressed. The resulting composite culture is, therefore, composed principally of the more rapidly developing strains, and only relatively small numbers of the more slowly developing strains are present, and consequently the resulting culture is inefficient 1133 for inoculating the seeds of plants which are infected by the normally slower growing organism. Furthermore, soil cultures prepared in accordance with the method suggested by Earp-Thomas have a high concentration of metabolic products of the bacteria developing therein, which products have a tendency to adversely affect the viability of the culture. Thus, the normal commercial shelf life of the Earp-Thomas product is much shorter than that proposed by the applicant.

The applicant has discovered that a composite culture containing a plurality of species of bacteria in predetermined numbers may be prepared by separately cultivating the organisms to obtain a desired number of bacteria of each species and thereafter mixing them with a moist powder base which is substantially free of bacterial nutrients in order to prevent subsequent overgrowth of the more rapidly developing species within the commercial inoculant thus produced. The applicant has been able by this process to produce a mixed culture in which the organisms of the several species may be present in predetermined relative concentrations. This method, which is recited by claims 16 and 26 as amended, is clearly not disclosed in any of the references cited by the Examiner. The Earp-Thomas patent is not pertinent, since it discloses a method in which the organisms are inoculated into a moist soil base having substantial quantities of a bacterial nutrient, thus readily permitting a rapidly developing species to outgrow a more slowly developing species when the culture is stored.

The Examiner has stated:

> "... It is submitted that where a product is required containing a plurality of species of which may not be cultivated together because of the inhibitory effect, it is elementary to grow them separately and combine them in the final mixture."

1134 The Examiner is apparently laboring under a misapprehension as to the meaning of "inhibitory" as employed by the applicant throughout the specification and claims. The term does not necessarily refer to mutual inhibition of bacteria as regards their growth in a common culture medium, although it may under certain conditions. It refers principally to the inhibitive effect of the organisms exerted on each other in respect to the power to produce proper nodulation on or fix nitrogen in the leguminous plant for which each is specific. Thus, one species growing in a mixed culture medium may have a tendency to outgrow another, but either or both may have an inhibitory effect on the other in a composite culture in regard to its ability to infect the host plant and to fix nitrogen therein. For example, if Rhizobium trifolii and Rhizobium japonicum are inoculated into a single culture medium, the trifolii, having a more rapid growth rate will usually outgrow the japonicum. However, if the seeds of a clover plant are inoculated with a mixed culture containing trifolii (not an alpha strain) and japonicum, the japonicum species will inhibit the trifolii in the latter's ability to infect the roots of the clover plant and to fix nitrogen therein. Consequently, there is no correlation between the "inhibitory" powers of the various species of the Rhizobia (as used in the present specification) and the rate of growth of each species in a common culture medium. The applicant has discovered that separate growth of the cultures and their mixing provides a method in which the approximate numbers of each species may be controlled and results in a product which will remain viable and effective over a long period of time. This process has not been suggested by any of the art of record and it is believed that the applicant is entitled to patent protection therefor.

1135 The Examiner has indicated in the last Office Action that claims 5 though 9, 12 through 15, and 21 through 25 were allowable. He has indicated, however, that the number of allowed claims should be reduced. Consequently, allowed claims 8, 9, 13, 21, and 22 have been withdrawn from the case, since it is believed that the remaining allowed claims are sufficient to define that feature of the applicant's invention directed to the noninhibitive

strains of the micro-organisms. They are all clearly patentably distinguishable from each other.

Claim 5 is directed to an inoculant comprising a plurality of selected cultures including Rhizobium trifolii alpha, which cultures are substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

Claim 6 is directed to a bacterial inoculant comprising Rhizobium trifolii alpha, Rhizobium meliloti alpha, and Rhizobium japonicum alpha.

Claim 7 is similar to claim 5, but specifically recites an inoculant comprising humus and Rhizobium trifolii alpha.

Claim 12 is directed broadly to an inoculant comprising a plurality of selected mutually noninhibitive strains of the Rhizobia. This claim thus includes other species of Rhizobia than those specified in claim 6.

Claim 14 is somewhat broader than claim 12 and recites that at least one of the strains present is not inhibited by the others in respect to its ability to fix nitrogen in the leguminous plant for which it is specific.

Claim 15 is similar to claim 12, but further points out that the bacteria of each strain are present in numbers of substantially the same order of magnitude.

1136 Claim 23 is similar to claim 5, but further recites that the inoculant has a moist powder base and that the numbers of the bacteria of each strain are of the same order of magnitude.

Claim 24 distinguishes from claim 14 in that it recites a moist powder base containing a substantially negligible concentration of bacterial nutrients.

Claim 25 recites a method of producing the inoculant set forth in the previously discussed claims which comprises first selecting the noninhibiting strains and subsequently cultivating them separately and mixing the resulting cultures to produce the composite inoculant.

From the above considerations, it is clear that all of the allowed claims are clearly patentably distinguishable from each other.

Amended claims 16 and 26 and newly added claim 27 are directed to the above-discussed method of preparing composite cultures which comprises separately cultivating the organisms and thereafter mixing them with a

moist powder base containing a substantially negligible concentration of bacterial nutrients. Newly added claims 28 and 29 are directed to a product resulting from the process which is clearly distinguishable from the art of record.

Claim 16 is the broad claim of this group, reciting a method in which each strain of several species of Rhizobium is separately cultivated before mixing with a moist powder base containing a substantially negligible concentration of bacterial nutrients.

Claim 26 is similar to claim 16, but further recites that the bacteria of each strain so mixed are present in the resulting inoculant in numbers of substantially the same order of magnitude.

1137 Claim 27 is similar to claim 16, but recites that the bacteria of each species is cultivated separately in suitable culture medium and thereafter mixed with the moist powder base in predetermined ratios, said powder being substantially free of added bacterial nutrients.

Claim 28 is directed to a composite culture prepared in accordance with the method recited in claim 26. The product suggested in Earp-Thomas clearly does not have the characteristics recited by this claim.

Claim 29 is dependent upon claim 28 and specifically recites that one of said species is Rhizobium trifolii.

It is submitted that neither of the patents to Hendrickson or Earp-Thomas anticipates any of the claims 16, 26, 27, 28, or 29. As pointed out above, Earp-Thomas contemplated that the bacteria be introduced into a soil base which contained considerable quantities of a carbohydrate or other bacterial nutrient to permit subsequent development of the organisms thereon. This is clearly an undesirable feature of his process and product; the applicant has distinguished from it, and it is believed that he is entitled to patent protection for his disclosure.

The patent to Hendrickson has been carefully considered and the Examiner is respectfully requested to point out wherein there is any disclosure of a method for producing a composite culture. The disclosure in this patent merely relates to a method of cultivating bacteria on a silica gel. The applicant is unable to find a disclosure of a method for producing a composite culture.

Discussion of the remaining references considered by

the Examiner in the last Office Action is believed to be unnecessary because of the withdrawal by the present amendment of certain claims in the application against 1138 which these references were applied. All of the claims as they now stand in the case are believed to be clearly in condition for allowance and such action is respectfully requested.

Respectfully submitted,
Thiess, Olson & Mecklenburger
Attorneys for Applicant.

Chicago, Illinois,
March 9, 1940.

1139

Div. 59 Room 4709 Serial No. 226,565

DEPARTMENT OF COMMERCE

UNITED STATES PATENT OFFICE

Washington

(Stamp) Mailed Apr. 1 1940

Varley Sherman Bond, Assor. April one, 1940

Your APPLICATION for a patent for an IMPROVEMENT in Bacterial Inoculant for Leguminous Plants filed 8-24-38 has been examined and ALLOWED with 14 claims.

The final fee, THIRTY DOLLARS, WITH $1 ADDITIONAL FOR EACH CLAIM ALLOWED IN EXCESS OF 20, must be paid not later than SIX MONTHS from the date of this present notice of allowance. If the final fee be not paid within that period, the patent will be withheld; SEE REVISED STATUTES, SECTION 4885 AS AMENDED BY ACT OF CONGRESS APPROVED AUGUST 9, 1939.

The office delivers patents upon the day of their date, on which date their term begins to run. The preparation of the patent for final signing and sealing will require about four weeks, and such work will not be begun until after payment of the necessary final fee.

When the final fee is paid, there should also be sent, DISTINCTLY AND PLAINLY WRITTEN, the name of the INVENTOR, TITLE OF THE INVENTION, AND SERIAL NUMBER AS ABOVE GIVEN, DATE OF ALLOWANCE (which is the date of this circular), DATE OF FILING, and, if assigned, the NAMES OF THE ASSIGNEES.

If it is desired to have the patent issue to an ASSIGNEE OR ASSIGNEES, an assignment containing a REQUEST to that effect, together with the FEE for recording the same, must be filed in this office on or before the date of payment of the final fee.

After issue of the patent, uncertified copies of the drawings and specifications may be purchased at the price of TEN CENTS EACH. The money should accompany the order. Postage stamps will not be received.

The final fee will NOT be received from other

IN REMITTING THE FINAL FEE GIVE THE SERIAL NUMBER AT THE HEAD OF THIS NOTICE.

UNCERTIFIED CHECKS WILL NOT BE ACCEPTED.

than the applicant, his assignee or attorney, or a party in interest as shown by the records of the Patent Office.

NOTICE.—WHEN THE NUMBER OF CLAIMS ALLOWED IS IN EXCESS OF 20, NO SUM LESS THAN $30 PLUS $1 ADDITIONAL FOR EACH CLAIM IN EXCESS OF TWENTY CAN BE ACCEPTED AS THE FINAL FEE.

Respectfully,
Conway P. Coe
Commissioner of Patents.

THIS APPLICATION
CANNOT BE RENEWED.
Thiess, Olson & Mecklenburger,
77 W. Washington St.
Chicago, Illinois.

1140 IN THE UNITED STATES PATENT OFFICE

Apr-13-40 200295 B-Check—30.00

(Stamp) Mail Division
Apr. 13, 1940
U. S. Patent Office

Application of
Varley Sherman Bond
Serial No. 226,565
Filed August 24, 1938
For Bacterial Inoculant
for Leguminous Plants
Assigned to Kalo Inoculant Company
Allowed April 1, 1940

Division 59
Room 4709

Honorable Commissioner of Patents
Washington, D. C.

Sir:

We enclose our check for $30.00 in payment of the final fee in the above-identified application. Please have the patent issue in the name of the Kalo Inoculant Company.

Respectfully,
Thiess, Olson & Mecklenburger

Chicago, Illinois
April 11, 1940

1149

DISTRICT COURT OF THE UNITED STATES
Southern District of Illinois

HONORABLE COMMISSIONER OF PATENTS,
Washington, D. C.

Sir:

In compliance with the Act of February 18, 1922 (42 Stat. L. 392), you are advised that there was filed on the 10th day of December, 1943, in this court an action, suit, or proceeding No. Civil Q-62, entitled:
Name Kala Inoculant Company, a corporation, Plaintiff,
Address Quincy, Illinois.

versus

Name Funk Brothers Seed Company, a corporation, Defendant,
Address Bloomington, Illinois.
brought upon the following patents:

	Patent No.	Date of Patent	Patentee
1	2,200,532	May 14, 1940	Varley Sherman Bond
2	.....	.....	.....
3	.....	.....	.....
4	.....	.....	.....
5	.....	.....	.....

In the above-entitled case, on the ..... day of ....., 193 , the following patents have been included by ..... (insert amendment, answer, cross bill, or other pleading):

	Patent No.	Date of Patent	Patentee
1	.....	.....	.....
2	.....	.....	.....
3	.....	.....	.....
4	.....	.....	.....
5	.....	.....	.....

In the above-entitled case the following decision has been rendered or decree issued:

.....

.....

IN WITNESS WHEREOF I have affixed my hand this 10th day of December, 1943, at Quincy, Illinois.

G. W. Schwaner
(sgd) G. W. Schwaner
Clerk of said Court.

1150 In The

DISTRICT COURT OF THE UNITED STATES
For The Southern District Of Illinois
Southern Division

Quincy, Illinois
December 10, 1943

The Attorney General,
Washington, D. C.,

Sir:

In compliance with your request of June 2, 1942, you are advised that there was filed on the 10th day of December, 1943, in this court, an action bearing general No. 62, Civil Action, entitled as follows:

Name Kalo Inoculant Company, a corporation, Plaintiff
Address Quincy, Illinois.

-vs-

Name Funk Brothers Seed Company, A corporation, Defendant.
Address Bloomington, Illinois.
brought upon the following patents:

Patent No.	Date of Patent	Patentee
2,200,532	May 14, 1940	Varley Sherman Bond

.....

.....

TRIAL CALENDAR STATUS

Summons issued December 10, 1943, returnable in twenty days.

.....

.....

In witness whereof, I have affixed my hand this 10th day of December, A. D. 1943, at Quincy, Illinois.

G. W. Schwaner
Clerk, U. S. District Court
Southern District of Illinois.

clf

1151 — 1938

CONTENTS

1. Application.....papers
2. Print 8-29-38
3. Rejection Nov. 9-1938
4. Amdt. A Apr. 29, 1939
5. Rejection Sep. 12, 1939
6. Sub Pow. Feb. 8, 1940
7. Amdt. B & Sheet Drg. Mar. 11, 1940
8. Notice of Suit Jan. 14-1944
9. Notice of Suit Mar. 6-1944
10. .....
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14. ..... 63 226565
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20. .....

U. S. Patent Office
Oct. 18, 1938
Division 59

DEFENDANT'S EXHIBIT 35.

1152 last analysis, the effect will be explainable in terms of physics and chemistry. But for the present we cover our ignorance by speaking of symbiosis and antagonism, according as the net effect of the association of organisms is beneficial or harmful to the rhizobia. The nodule bacteria have undoubtedly existed in the soil and tissues of the Leguminosae for ages. Why are they not more abundant and more widely distributed? Perhaps the answer is that the antagonism of other soil microörganisms holds them in check. As early as 1899b Nobbe and Hiltner suggested that the competition between rhizobia and the rest of the soil flora results in harm to the rhizobia; they implicated both bacteria and fungi in the opposition. Sackett, 1906, in a laboratory study of associated growth of *B. ramosus* and *Ps. radicicola,* observed that multiplication of the latter is greatly inhibited. Simon, 1907, apparently the first to study the rhizobia in natural soil, reported that the soil flora is definitely antagonistic to the nodule organisms, and in a later paper, 1911, asserted that the presence of fungi is particularly harmful. From the fact that rhizobia grow better in sterilized soil than in natural unsterilized soil, Duggar and Prucha, 1912, argued that there is an antagonistic action of the general soil flora toward the rhizobia. Kühn, 1911, on finding poor results with certain commercial cultures on seeds in rich humus soil, reasoned that competing microörganisms in such a soil destroy the rhizobia. Other investigators, however, are only willing to grant that certain bacteria of the soil flora are harmful. Kellerman and Robinson, 1906, reported an organism resembling *B. coli* to be inhibitory to the rhizobia. A year later Kellerman and Fawcett, 1907, found that *B. ochraceus* in a liquid medium has no effect upon nodule bacteria, whereas an organism like *B. coli* is distinctly harmful. In soil, however, no harmful effect is noted. Kalantarov, 1914, showed that *Bacillus mycoides* and *Bacterium fluorescens liquefaciens* neither in soil nor in culture media influence the nodule bacteria. On the other hand, he observed that *Bacillus mesentericus*

vulgatus and *Bacterium coli communis* are strongly antagonistic. Alicante, 1926, found a number of organisms non-injurious to the rhizobia; namely *B. radiobacter, Azotobacter chroococcum, B. prodigiosus, B. capsulatus, B. subtilis, B. mesentericus,* a pink yeast, and some molds. *A mixture of nodule bacteria from pea, clover, cowpea, and soybean nodules are likewise not mutually antagonistic.*

While at Wisconsin, *Konishi,* 1931, obtained some interesting data on the relation of certain soil bacteria to the rhizobia. He isolated several unidentified organisms from soil and tested them out with rhizobia in liquid medium, with and without $CaCO_3$, and also in soil. He found inherent differences in the degree of antagonism exhibited by the cultures and also differences according to the conditions of the experiment. In general, the effects were most severe in liquid media without $CaCO_3$ and least severe in soil. Certain known cultures of soil bacteria were tested and also found most injurious in liquid. For example, *B. subtilis* inhibits *Rh. meliloti, Rh. leguminosarum,* and *Rh. japonicum* in liquid cultures, but not in soil. *B. coli* likewise is without effect on *Rh. meliloti* in soil. The other organisms tested, *B. mycoides, B. megatherium, B. prodigiosus, B. fluorescens,* and *B. aerogenes,* are also harmless in soil.

So much for the antagonistic or neutral side to the competition. There remain to be considered a few reports on the beneficial effects of certain organisms upon the rhizobia. Hiltner, 1907, stated that the presence of another organism

DEFENDANT'S EX. 36.

1153 exhibits slower and less profuse growth and an alkaline reaction, rarely, if ever, an acid reaction. Associated with these characters is a difference in the growth of the organisms in milk. The first group produces a serum zone without coagulation, while the second group gives no macroscopic change in the milk. To the first group belong the organisms from alfalfa, clover, pea, bean, and Dalea, and to the second, the organisms of soybean, cowpea, and lupine.

As the study of these organisms has continued, it has become increasingly apparent that these two large groups can be further subdivided on the basis of cultural and physiological characters. These groupings follow the lines of the cross-inoculation groups; and although the differences are not as distinct as those separating the two main groups, it is evident that the organisms of the various cross-inoculation groups are not identical, either culturally or physiologically. The work of Müller and Stapp, 1925, is the most thorough and exhaustive yet published, but the reports of Simon, 1911, and of Prucha, 1915, with the organism from pea; of Wilson, 1917, and Wright, 1925a, with the organism from soybean; of Stevens, 1925a, with the organism from alfalfa; of Gangulee, 1926a and b, with the organism from *Crotalaria juncea;* of Whiting, Fred, and Helz, 1926, with the organism from Dalea; and of Eckhardt, Baldwin, and Fred, 1931, with the organism from *Lupinus* deserve mention.

Studies on the fermentative characters and gelatin liquefaction by the various rhizobia have been carried out by a number of workers recently. It has been found that significant differences do exist; these have been stated fully in Chapter 6 and will be introduced wherever useful in the species characterization which will be given later in this chapter. In some cases the variation between the reaction of known strains of the same rhizobium is so great that apparent differences between cross-inoculation groups cannot be urged too far. One would hesitate to identify an unknown culture of rhizobia entirely on the basis of fer-

mentative characters; yet the authors feel that the fermentation reactions are valuable aids in species characterization and should be used as far as may be.

Serological reactions. With the development of serological reactions by workers with animal pathogens and their general adoption as an aid in species identification, a number of studies were made on the antigenic specificity of the rhizobia. The early results of Zipfel, 1911; Krüger, 1914; Klimmer and Krüger, 1914; Simon, 1914; Vogel and Zipfel, 1921; Klimmer, 1922; and Aso and Ohkawara, 1926; as well as the more recent studies of Walker, 1928, indicated that certain serological reactions, particularly the agglutination test, might be used to separate the various species of rhizobia. In each instance one, or at most a very few, strains of each species were tested. The results with such a limited number of strains render of little value the sweeping conclusions which were often drawn. The fact that in no instance was cross agglutination observed between different species is probably satisfactory evidence for the conclusion that the species are serologically distinct. On the other hand, the assumption by several investigators that all strains of a species are serologically identical has little basis in fact.

Several serological groups have been recognized within the species by a number of workers: Bialosuknia, 1923; Bialosuknia and Klott, 1923; Stevens, 1923 and 1925a; Wright, 1925a; Ohkawara and Yoshida, 1925 (see Jimbo, 1930); Israilsky, 1929; and Jimbo, 1930. The work of Stevens, 1923, is the most exten-

1154 And afterwards, to-wit: on the 27th day of September, A. D. 1945, the following further proceedings were had in said Court in said cause and were entered of record, to-wit:

1155 IN THE DISTRICT COURT OF THE UNITED STATES
Southern District of Illinois
Southern Division

Kalo Inoculant Company, Plaintiff, *vs.* Funk Brothers Seed Company, Defendant.	Civil Action No. Q-62

MEMORANDUM OF HOLDINGS

With some reluctance I find that the claims in suit of the Bond patent are invalid under our patent statute because they do not involve the invention or discovery of "any new and useful art, machine, manufacture, or composition of matter." I say, "with reluctance," because I was impressed at the trial with the very great diligence and ingenuity of Mr. Bond and with the results he obtained. His work is not to be minimized. Notwithstanding the value of his work, however, I feel quite positive that it cannot be classified under any of the subjects covered by the Act and must, therefore, be held nonpatentable. I deem this holding to be in harmony with the weight of authority and not in conflict with the decision in Dennis v. Pitner. This is sufficient to dispose of the case, but if it is desired by counsel that I pass upon other questions at issue my holding will be, as follows:—

1. If valid Legume-Aid infringes.
2. Finding in favor of Plaintiff on issue of "unclean hands."
3. Bond is the sole inventor.

Plaintiff has limited its charges of infringement in this proceeding to those arising under claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 and these I have held invalid. The other claims I deem not to be at issue, and the defendant in this proceeding is not entitled to any relief by way of declaratory

judgment concerning them. The counterclaim will, therefore, be dismissed.

This memorandum of holdings is intended as a guide in the preparation of findings. Counsel for defendant may prepare and submit (first to opposing counsel) findings of fact, conclusions of law and decree.

(signed) Chas. G. Briggle
U. S. District Judge

Endorsed, Filed September 27, 1945 G. W. Schwaner, Clerk.

1156 And afterwards, to-wit: on the 10th day of October, A. D. 1945, the following further proceedings were had in said Court in said cause and were entered of record, to-wit:

1157 IN THE DISTRICT COURT OF THE UNITED STATES
For the Southern District of Illinois
Southern Division

Kalo Inoculant Company, Plaintiff,
vs.
Funk Brothers Seed Co., Defendant.
} Civil Action No. Q-62

FINDINGS OF FACT AND CONCLUSIONS OF LAW

This cause having come on to be heard regularly by the Court sitting without a jury and the same having been submitted upon the pleadings, all of the proceedings had herein and the evidence introduced at the trial, oral argument having been had in open Court and briefs filed by counsel for all the parties; and the Court having taken the same under advisement and indicated to counsel in a Memorandum of Holdings its decision as to the issues involved, the Court does hereby make and file its Findings of Fact and Conclusions of Law pursuant to the provisions of Rule 52 of the Federal Rules of Civil Procedure.

1.

This is a patent infringement suit brought by the Kalo Inoculant Company, an Illinois corporation, against Funk Brothers Seed Company, also an Illinois corporation, based upon U. S. Letters Patent No. 2,200,532 granted May 14, 1940, to Varley Sherman Bond, Quincy, Illinois, assignor to Kalo Inoculant Company, Quincy, Illinois, entitled "Bacterial Inoculant for Leguminous Plants."

2.

The patent in suit contains some fourteen claims in all, Claims 9, 10, 11 and 12 being process or method claims. All of the other claims are product or article claims. By stipulation of counsel the Plaintiff limited its charge of infringement to those arising under product Claims 1, 3, 4, 5, 6, 7, 8, 13 and 14.

3.

By amendment the Defendant further averred that 1158 the patentee had made false statements to the Patent Office pending the application for the patent here involved and that as a consequence the Plaintiff in this action does not come into Court with clean hands and should, therefore, be held to be without any remedy. The answer further embraced a counterclaim under the declaratory judgment Act of 1934, Section 274(d) of the Judicial Code of the United States, 28 U.S.C.A. 400 and prayed for a judgment declaring the Bond patent in suit No. 2,200,532 wholly null and void and invalid in law as to all claims thereof, and, if valid, not infringed by the Defendant. To this counterclaim the Plaintiff in due season filed its reply traversing the issues raised under the same.

4.

The specific acts of infringement complained of took place in the City of Bloomington, Illinois, in the Southern Division of the Southern District of Illinois, within the jurisdiction of this Court, and subsequent to the issue of the patent in suit and consisted of the sale by the Defendant of record, Funk Brothers Seed Company, of a bac-

terial inoculant for leguminous plants under the trade mark or name of "Legume-Aid."

5.

The bacterial inoculant sold under the trade mark or trade name "Legume-Aid," is a product manufactured and sold by The Agricultural Laboratories, Inc. of Columbus, Ohio, a corporation of the State of Ohio, which company, openly and avowedly, assumed the defense of this action and controlled the same though said company was not made a formal party Defendant of record. The accused product here involved was manufactured by The Agricultural Laboratories, Inc. and sold by it to the defendant of record, Funk Brothers Seed Company, which Defendant in turn sold the same to its customers within the jurisdiction of this Court, and within the period of six years next preceding the filing of the Bill of Complaint herein and subsequent to the date of issue of said Bond patent in suit No. 2,200,532.

6.

Jurisdiction of the Court is based upon the Patent
1159 Laws of the United States and no issue was raised by either party as to the same.

7.

The Plaintiff is the owner of the Bond patent in suit No. 2,200,532 and has been the owner of the same at all times since the issuance of the patent on May 14, 1940.

8.

The Bond patent in suit is entitled "Bacterial Inoculant for Leguminous Plants" and in general relates to a bacterial culture for use in the inoculation of leguminous plants. It discloses and claims a composite culture in which there is mixed a plurality of different species of bacteria belonging to the general genus known as Rhizobium in a common powder base. The claimed inventive thought of the Bond patent rests primarily upon the fact that the separate cultures of these different species in the composite inoculant are unaffected by the others in respect

to their ability to fix nitrogen in the leguminous plant for which each is specific.

9.

There are certain species of the Rhizobium bacteria that are specific to one group of leguminous plants and other species of the same genus Rhizobium that are specific to another group of leguminous plants. These groups of leguminous plants that may be infected by a single species or strain of the genus Rhizobium bacteria are known as cross inoculation groups.

10.

These bacteria of the genus Rhizobia which are capable of infecting leguminous plants so that nitrogen may be fixed are widely spread in nature. The mechanism of this nitrogen fixation is concededly not understood at the present time, but it has been known for a number of years that it can only be carried out when the plant grows in symbiotic relationship with certain bacteria of the genus Rhizobia. Plants belonging to the genus Leguminosae which are capable of symbiotic relationship with the Rhizobia are peas, beans, alfalfa, red clover, white clover, vetch, 1160 lupines and the like. The bacteria infect the roots of these plants in the soil and cause the formation of so-called nodules, in which it is presumed that the nitrogen fixation in the plant occurs.

11.

It was well known in the art long before the claimed invention or discovery of Bond that the genus Rhizobium bacteria possessed this nitrogen fixation characteristic when a certain species or strain of the same was applied in symbiotic relationship to the leguminous plant of the cross inoculation group to which the particular species or strain was specific. Also long before the advent of Bond in this field, manufacturers of inoculants had taken these bacteria as found widely scattered in nature where they performed this nodulation or nitrogen fixation and by a laboratory method of culture increased the number of the bacteria without in any way changing their form, shape or characteristics physiologically or otherwise and then placed

them in a powder or liquid base or carrying medium and packaged the same for sale to and use by agriculturists, farmers and others in the inoculation of leguminous plants.

12.

The patentee Bond did not invent any new strain or species of the Rhizobium bacteria but he discovered the fact that among the bacteria found widely spread in nature there existed both mutually inhibitive and mutually non-inhibitive strains which can be selected and isolated and when so selected and isolated perform the same function that they do in nature. The method of culture of single strains of these bacteria set forth in the Bond patent in suit was well known in the art long before Bond's claimed invention or discovery. His method of placing the bacteria in the carrying medium or base was a conventional and well known method of producing cultures prior to his claimed invention. The base or carrying medium of the Bond inoculant as disclosed and claimed in the patent in suit was likewise well known and not claimed as any invention of the patentee.

13.

The broad basis or theory of the claimed invention
1161 or discovery disclosed and claimed in the Bond patent in suit is the placing in a common base or carrying medium two or more different species of bacteria with the result that one species in the composite culture produces or has no effect on the other species in the matter of the fixation of nitrogen in the host plant when applied as an inoculant. This is stated by the patentee in the patent itself as follows:

> "It has heretofore been considered impractical to prepare a composite inoculant containing organisms which will cause nodulation on more than one of the cross inoculation groups. This has not been done because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen fixation by the plant and the organism was inhibited or even prevented." (See lines 70-75, column 1 and lines 1-4, column 2, page 2, of Bond patent in suit.)

14.

The Court finds that the fact of inhibition or lack of inhibition is a work of nature and nothing that the patentee did or disclosed in his patent brought about this state of inhibition or lack of inhibition in the composite culture of the patent. The patentee ascertained by well known routine tests the existence or nonexistence of this inhibitive characteristic.

15.

The Court further finds that all of the claims in issue of the Bond patent in suit relied upon by the Plaintiff do not involve the invention or discovery of "any new and useful art, machine, manufacture or composition of matter" within the contemplation of the patent laws of the United States.

16.

While the foregoing Findings of Fact are sufficient to dispose of the case, the Court further finds that the product sold by the Defendant of record, Funk Brothers Seed Company, and manufactured by the Agricultural Laboratories, Inc. under the trademark or trade name of "Legume-Aid" is a composite inoculant for leguminous plants consisting of a plurality of selected cultures of bacteria, comprising at least two species or strains of bacteria of the genus Rhizobium, which do not affect one another in their
1162 respective abilities to fix nitrogen in association with leguminous plants for which each species or strain is specific.

17.

The Court further finds that in the composite inoculant of the make of The Agricultural Laboratories, Inc. sold by the Defendant of record here, the number of bacteria put in the common base or carrying medium was approximately ten million of the alfalfa bacteria per standard unit and fourteen million of clover bacteria per standard unit.

18.

The Court further finds as a matter of fact that if the claims in issue of the Bond patent in suit relied upon by

the Plaintiff as infringed, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 were valid in law, the accused composite culture sold by the Defendant of record Funk Brothers Seed Company, under the trade mark or name "Legume-Aid" would constitute an infringement of each and all of said claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 of said Bond patent in suit No. 2,200,532.

19.

The Court further finds that prior to the claimed invention of the Bond patent in suit substantial portions of the available literature indicated that mixed cultures for field legumes were generally unsatisfactory and that different strains or species of the Rhizobium bacteria exhibited an inhibitory effect on each other when mixed in a common base or carrier, that Mr. Bond believed in the existence of this inhibitory effect at the time he instituted his research and acted in complete good faith when he stated in his application for the patent in suit that it had been considered impractical to prepare a composite culture inoculant which would cause nodulation on more than one of the cross inoculation groups because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic fixation by the plant and the organism was inhibited or even prevented, and that plaintiff is not guilty of the charge of unclean hands.

1163

20.

The Court further finds that while the thought or general idea of the desirability of a composite culture was suggested to the patentee Bond by the witness Myron E. Lusk, the discovery of the concrete means of the solution of the problem, if any, was the act of the patentee Varley Sherman Bond and the invention or discovery, if any, was the sole act of said patentee and the said Myron E. Lusk was not a co-inventor.

21.

The Court furthef finds that in view of the finding of the claimed invention of the Bond patent in suit as to the claims in issue being not patentable invention, it is not

considered necessary to pass upon the questions or issues of the prior art patents, publications, uses and knowledge constituting any anticipation.

22.

The Court further finds that the Plaintiff limited its charges of infringement in this proceeding to those arising under claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 and the Court finding that each and all of these claims are invalid under the patent statute of the United States, the other claims of the Bond patent in suit are, therefore, not at issue, with the result that the Defendant is not entitled to any relief by way of declaratory judgment concerning said other claims under the counterclaim of the Defendant.

CONCLUSIONS OF LAW

1.

From all of the above the Court concludes as a matter of law that the Bond patent No. 2,200,532 as to each and every of the claims thereof in suit herein, to-wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 are null and void as not involving any invention or discovery of any new and useful art, machine, manufacture or composition of matter.

2.

That the Bond patent No. 2,200,532 as to each and every of the claims thereof in suit herein, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 do not disclose patentable invention.

3.

That the remaining claims of said Bond patent No.
1164 2,200,532 to wit, claims 2, 9, 10, 11 and 12 are not at issue in these proceedings.

4.

That the Defendant, Funk Brothers Seed Company, is entitled to a judgment or decree dismissing Plaintiff's com-

plaint for want of equity as to each and all of the claims of the Bond patent in suit No. 2,200,532 in issue herein, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14.

5.

That the Plaintiff, Kalo Inoculant Company, is entitled to a judgment or decree dismissing the counterclaim of the Defendant herein as to the remaining claims of the Bond patent No. 2,200,532, to wit, claims 2, 9, 10, 11 and 12 thereof, as not involving any justiciable issue under the Federal Declaratory Judgment Act.

6.

That the Defendant, Funk Brothers Seed Company, is entitled to have and recover from the Plaintiff two-thirds of the costs to be taxed by the Clerk of the Court, the balance of the costs to be taxed against defendant.

(signed) Chas. G. Briggle,
United States District Judge.

Endorsed, Filed,
October 10, 1945,
G. W. Schwaner, Clerk.

1165 And afterwards, to-wit: on the 10th day of October 1945, A. D. 1945, the following further proceedings were had in said Court in said cause and were entered of record, to-wit:

1166 IN THE DISTRICT COURT OF THE UNITED STATES,
For the Southern District of Illinois
Southern Division

Kalo Inoculant Company, Plaintiff, *vs.* Funk Brothers Seed Co., Defendant.	Civil Action No. Q-62

JUDGMENT

This cause having come on to be heard on the pleadings and proof and the Court having taken the same under advisement and having made and filed herein special Findings of Fact and Conclusions of Law:

And now upon the foregoing Findings of Fact and Conclusions of Law filed herein it is

ORDERED, ADJUDGED AND DECREED

1. That the Bill of Complaint herein be and the same hereby is dismissed on the merits for want of equity.

2. That the counterclaim of the Defendant filed herein be and the same hereby is dismissed as not involving any justiciable issue between the parties hereto under the Federal Declaratory Judgment Act, not otherwise disposed of.

3. That the costs herein be taxed two-thirds against plaintiff and one-third against defendant.

Dated this 10th day of October, 1945.

(signed) Chas. G. Briggle
United States District Judge

Endorsed, Filed,
October 10, 1945,
G. W. Schwaner, Clerk.

1167 And afterwards, to-wit: on the 2nd day of January, 1946, there was filed in the office of the Clerk of said Court in said cause a certain Notice of Appeal which said Notice of Appeal was and is in the words and figures following, to-wit:

1168 IN THE DISTRICT COURT OF THE UNITED STATES
For the Southern District of Illinois
Southern Division

Kalo Inoculant Company, Plaintiff, *vs.* Funk Brothers Seed Co., Defendant.	Civil Action No. Q-62

NOTICE OF APPEAL

Notice is hereby given that Kalo Inoculant Company, Plaintiff, hereby appeals to the United States Circuit Court of Appeals for the Seventh Circuit from the judgment entered in this action on October 10, 1945.

A bond for costs on appeal is filed herewith.

(signed) Chester A. Groves,
Attorney for Plaintiff
W.C.U. Building, Quincy, Illinois
(signed) J. Bernhard Thiess
(signed) Sidney Newman
(signed) M. Hudson Rathburn
Of Counsel
77 W. Washington Street,
Chicago, Illinois.

Endorsed, Filed,
January 2, 1946,
G. W. Schwaner, Clerk.

1169 And afterwards, to-wit: on the 8th day of February, A. D. 1946, there was filed in the office of the Clerk of said Court in said cause a certain Plaintiff's Statement of Points on Its Appeal which said Plaintiff's Statement of Points on its Appeal was and is in the words and figures following, to-wit:

1170 IN THE DISTRICT COURT OF THE UNITED STATES
Southern District of Illinois
Southern Division

Kalo Inoculant Company, a corporation, Plaintiff, *vs.* Funk Brothers Seed Company, a corporation, Defendant.	Civil Action No. Q-62

PLAINTIFF'S STATEMENT OF POINTS ON ITS APPEAL

The Kalo Inoculant Company, plaintiff, hereby states that the following are the points involved on its appeal from the judgment on its complaint herein:

1. The Court erred in finding that all of the claims in issue of the Bond patent in suit do not involve the invention or discovery of "any new and useful art, machine, manufacture, or composition of matter" within the contemplation of the patent laws of the United States.

2. The Court erred in not finding that all of the claims in issue of the Bond patent in suit relied upon by the plaintiff involve the invention or discovery of a new and useful composition of matter within the contemplation of the patent laws of the United States.

3. The Court erred in not finding that claims 13 and 14 of the Bond patent in suit are not limited to a composition of matter or composite culture containing mutually non-inhibitive strains of root nodule bacteria.

1171 4. The Court erred in concluding as a matter of law that the Bond patent, as to each and every one of the claims in suit, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14, is null and void as not involving any invention or discovery of any new and useful art, machine, manufacture or composition of matter.

5. The Court erred in concluding that the Bond patent, as to each and every one of the claims thereof in suit, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14, does not disclose patentable invention.

6. The Court erred in not finding and concluding that all of the claims in issue of the Bond patent in suit, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 are good and valid in law.

7. The Court erred in dismissing plaintiff's Complaint for want of equity.

(signed) Thiess, Olson & Mecklenburger
Attorneys for Plaintiff.

Receipt of a copy of the foregoing Statement of Points on appeal is hereby acknowledged this 31st day of January, 1946.

(signed) Toulmin & Toulmin
Attorneys for Defendant.

Endorsed, Filed,
February 8, 1946,
G. W. Schwaner, Clerk.

1172 And afterwards, to-wit: on the 2nd day of January, A. D. 1946, there was filed in the office of the Clerk of said Court in said cause a certain Bond for Costs which Bond for Costs was and is in the words and figures following, to-wit:

1173 UNITED STATES DISTRICT COURT
Southern District of Illinois
Southern Division

Kaló Inoculant Company, Plaintiff, *vs.* Funk Brothers Seed Company, Defendant.	Civil Action No. Q-62

BOND FOR COSTS

Know All Men by These Presents that Kalo Inoculant Company, an Illinois Corporation, as principal, and Hartford Accident and Indemnity Company, a corporation of the State of Connecticut, as surety, are held and firmly bound unto Funk Brothers Seed Company, an Illinois corporation, in the full and just sum of Two Hundred Fifty Dollars ($250.00) to be paid to the said Funk Brothers

Seed Company, its successors or assigns, to which payment well and truly to be made, we bind ourselves, our successors and assigns, jointly and severally, by these presents.

Sealed with our seals and dated this 27th day of December, 1945.

Whereas, on the 10th day of October, 1945, in an action in the United States District Court for the Southern District of Illinois, Southern Division, between Kalo Inoculant Company, as plaintiff, and Funk Brothers Seed Company, as defendant, a judgment was entered against the Kalo Inoculant Company dismissing its complaint on the Bond patent No. 2,200,532, dated May 14, 1940, and taxing two-thirds of the costs against the plaintiff, and the said Kalo Inoculant Company has filed a notice of appeal to the
1174 United States Circuit Court of Appeals for the Seventh Circuit from said judgment.

Now, the condition of this obligation is such that if the Kalo Inoculant Company shall prosecute its appeal to effect and shall pay all its costs on said appeal if said appeal is dismissed, or if the judgment from which said plaintiff appeals is affirmed, or such costs as the Circuit Court of Appeals may award if the judgment from which said plaintiff appeals is modified, then this obligation shall be void; otherwise to remain in full force and effect.

Kalo Inoculant Company
(seal) By (signed) M. E. Lusk
Its President

Hartford Accident and Indemnity Company
By (signed) Frank J. Souhey
Attorney in Fact
(seal)

Endorsed, Filed,
January 2, 1946,
G. W. Schwaner, Clerk.

1175 And afterwards, to-wit: on the 8th day of January, A. D. 1946, there was filed in the office of the Clerk of said Court in said cause a Certain Notice of Cross-Appeal which said Notice of Cross-Appeal was and is in the words and figures following, to-wit:

1176 IN THE DISTRICT COURT OF THE UNITED STATES
For the Southern District of Illinois
Southern Division

Kalo Inoculant Company, a corporation, Plaintiff, *vs.* Funk Brothers Seed Company, a corporation, Defendant.	Civil Action No. Q-62

NOTICE OF CROSS-APPEAL

Notice is hereby given that Funk Brothers Seed Company, Defendant, hereby cross-appeals to the United States Circuit Court of Appeals for the Seventh Circuit, from such portions of the judgment entered in this action on October 10, 1945, as constitute an aggrievement of Defendant, to-wit:

1. As to so much of the judgment directed to a finding that the accused product sold by the Defendant, did constitute an infringement of each and all of the claims in suit if the said claims were valid in law.

1177 2. As to so much of the judgment directed to a finding that it is not considered necessary to pass upon the questions or issues of the prior art patents, publications, uses and knowledge, constituting any anticipation of the Bond patent, in view of the finding of invalidity of the claims in suit on other grounds.

3. As to so much of the judgment directed to a finding that the Defendant is not entitled to any relief by way of Declaratory Judgment under the counterclaim of the Defendant.

A bond for costs on cross-appeal is filed herewith.

(signed) Toulmin & Toulmin
Attorneys for Defendant.

(signed) H. A. Toulmin, Jr.
(signed) D. C. Staley
(signed) David Davis
(signed) Harold W. Lewis
Solicitors and Of Counsel
for the Defendant.

Endorsed, Filed,
January 8, 1946,
G. W. Schwaner, Clerk.

1178 And afterwards, to-wit: on the 6th day of February, A. D. 1946, there was filed in the office of the Clerk of said Court in said cause a certain Statement of Points to be Relied Upon by the Defendant-Appellant on Cross-Appeal of this Action which said Statement of Points was and is in the words and figures following, to-wit:

1179 IN THE DISTRICT COURT OF THE UNITED STATES
Southern District of Illinois
Southern Division

Kalo Inoculant Company, a corporation, Plaintiff,	Civil Action No. Q-62
v.	
Funk Brothers Seed Company, a corporation, Defendant.	

STATEMENT OF POINTS TO BE RELIED UPON BY THE DEFENDANT-APPELLANT ON CROSS-APPEAL OF THIS ACTION.

Now comes the Defendant, Appellee; and Appellant on cross-appeal, Funk Brothers Seed Company, and pursuant to Rule 75(d) of the Federal Rules of Civil Procedure and Rule 9(1) of the Rules of the Circuit Court of Appeals for the Seventh Circuit, files this, its statement of points on which it intends to rely on the cross-appeal in this case.

1. The District Court erred as a matter of law in finding and holding that the product sold by the Defendant of record, Funk Brothers Seed Company, and manufactured by Agricultural Laboratories, Inc. under the trade-mark or trade-name of "LEGUME-AID" is a composite inoculant for leguminous plants consisting of a plurality of selected cultures of bacteria, comprising at least two species or strains of bacteria of the genus Rhizobium of different cross-inoculation groups which
1180 do not affect one another in their respective abilities to fix nitrogen in association with leguminous plants for which each species or strain is specific.

2. The District Court erred as a matter of law in finding and holding that as a fact there exists in nature mutually inhibitive and mutually non-inhibitive strains of Rhizobium bacteria.

3. The District Court erred as a matter of law in its finding and holding that if the claims in issue of the Bond patent in suit, #2,200,532, relied upon by the Plaintiff as infringed, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 were valid in law, the accused composite product sold by the Defendant of record under the trade-mark or trade-name "LEGUME-AID" would constitute an infringement of each and all of the said claims.

4. The District Court erred as a matter of law in failing to find and hold that if the claims in issue of the Bond patent in suit, #2,200,532, relied upon by the Plaintiff as infringed, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 were valid in law, the accused composite product sold by the Defendant of record under the trade-mark or trade-name "LEGUME-AID" would not constitute any infringement of either or all of said claims.

5. The District Court erred as a matter of law in holding that since the Plaintiff limited its charges of infringement to those arising under claims 1, 3, 4, 1181 5, 6, 7, 8, 13 and 14 of the Bond patent #2,200,532, that the other claims of the Bond patent in suit are, therefore, not at issue in the proceedings, whereas he should have held all of the claims of the patent to be invalid.

6. The District Court erred as a matter of law in failing to find and hold that claims 2, 9, 10, 11 and 12 of the Bond patent in suit, #2,200,532 are at issue in the proceedings, and were invalid.

7. The District Court erred as a matter of law in finding and holding that since all of the claims placed at issue by the Plaintiff, to wit, claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 are declared to be invalid under the Patent Statute of the United States, that the Defendant is not entitled to any relief by way of declaratory judgment concerning other claims of the patent in suit under the counterclaim of the Defendant.

8. The District Court erred as a matter of law in holding that the Plaintiff is entitled to a judgment or decree dismissing the counterclaim of the Defendant as to claims 2, 9, 10, 11 and 12 as not involving any justici-

able issue under the Federal Declaratory Judgment Act.

9. The District Court erred as a matter of law in failing to find and hold that the claims of the Bond patent in suit, #2,200,532, not specifically placed at issue by the Plaintiff, to wit, claims 2, 9, 10, 11 and 12, in-
1182 volve justiciable issues under the Federal Declaratory Judgment Act under the counterclaim of the Defendant and in failing to pass upon such issues.

10. The District Court erred as a matter of law in dismissing the counterclaim of the Defendant under the Federal Declaratory Judgment Act as to any or all of the claims of the patent in suit.

11. The District Court erred as a matter of law in dismissing the counterclaim of the Defendant under the Federal Declaratory Judgment Act as to any or all of the claims of the patent in suit without passing upon the questions or issues of the prior art patents, publications, uses and knowledge constituting anticipation of the patent in suit, and in not holding that all the claims in the patent in suit were invalid.

12. The District Court erred as a matter of law in not passing upon the questions or issues of the prior art patents, publications, uses and knowledge constituting anticipation of the patent in suit in view of Defendant's counterclaim under the Federal Declaratory Judgment Act.

13. The District Court erred as a matter of law in finding and holding that in view of the finding that the claimed invention of the Bond patent in suit, as to the claims in issue being not patentable invention, it is not considered necessary to pass upon the questions or issues of the prior art patents, publications, uses
1183 and knowledge constituting any anticipation of the patents in suit.

14. The District Court erred as a matter of law in dismissing the counterclaim of the Defendant as not involving any justiciable issue between the parties under the Federal Declaratory Judgment Act.

15. The District Court erred as a matter of law in finding and holding that the patentee Bond had reason to believe in the existence of the inhibitory effect between different strains or species of the Rhizobium bacteria and acted in complete good faith when he stated in his application for the patent in suit that it had been con-

sidered impractical to prepare a composite inoculant which would cause nodulation on more than one of the cross-inoculation groups because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen fixation by the plant and the organism was inhibited or even prevented, and that Plaintiff is not guilty of the charge of unclean hands, and in failing to find and hold that the patentee did not act in complete good faith when he made the aforementioned statements, and that Plaintiff is guilty of the charge of unclean hands.

16. The District Court erred as a matter of law in finding and holding that while the thought or general idea of the desirability of a composite culture was suggested to the patentee Bond by the witness, Myron
1184 E. Lusk, the discovery of the concrete means of the solution of the problem, if any, was the act of the patentee Varley Sherman Bond, and the invention or discovery, if any, was the sole act of the patentee, and in failing to find and hold that the said Myron E. Lusk was a co-inventor of the invention or discovery, if any, and the patent to be invalid in law as the result thereof.

Respectfully submitted,
(Signed) Toulmin & Toulmin
Attorneys for Defendant.

Receipt of a copy of the foregoing Statement of Points on Cross-appeal is hereby acknowledged this 4th day of February, 1946.

(Signed) Thiess, Olson & Mecklenburger
Attorneys for Plaintiff.

Endorsed, filed,
February 6, 1946,
G. W. Schwaner, Clerk.

1185 And afterwards, to-wit: on the 8th day of January, 1946, there was filed in the office of the Clerk of said Court in said cause a certain Cost Bond on Cross-Appeal which said Cost Bond on Cross-Appeal was and is in the words and figures following, to-wit:

1186 IN THE DISTRICT COURT OF THE UNITED STATES
For the Southern District of Illinois
Southern Division

Kalo Inoculant Company, a corporation, Plaintiff, v. Funk Brothers Seed Company, a corporation, Defendant.	Civil Action No. Q-62

COST BOND ON CROSS-APPEAL.

KNOW ALL MEN BY THESE PRESENTS, that we, Funk Brothers Seed Company, an Illinois corporation, with its principal place of business in Bloomington, Illinois, as principal, and The Indemnity Insurance Company of North America, a corporation, organized and existing under the laws of the State of Pennsylvania, with its principal place of business in Philadelphia, Pennsylvania, and duly authorized to transact a general surety business in the State of Illinois, as surety, are held and firmly
1187 bound unto Kalo Inoculant Company, an Illinois corporation, with its principal place of business in Quincy, Illinois, its attorneys, executors, administrators, successors or assigns, in the full and just sum of Two Hundred Fifty ($250) Dollars, to which payment, well and truly to be made, we bind ourselves, our successors, executors, administrators or assigns, jointly and severally by these presents.

Sealed with our seals and dated this 7th day of January, 1946.

WHEREAS, lately on October 10, 1945, in an action depending in the United States District Court for the Southern District of Illinois, Southern Division, between Kalo Inoculant Company, Plaintiff, and Funk Brothers Seed Company, Defendant, a final judgment or decree was rendered against the said Kalo Inoculant Company, and the said Kalo Inoculant Company, having filed a Notice of Appeal from such judgment or decree to the United States Circuit Court of Appeals for the Seventh Circuit,

pursuant to Rule 73 of the Federal Rules of Civil Procedure; and the said Funk Brothers Seed Company having filed a Notice of Cross-Appeal from such portions of said judgment or decree as constitute an aggrievement of Defendant, to the United States Circuit Court of Appeals for the Seventh Circuit;

1188 Now, the condition of this obligation is such, that if the said Funk Brothers Seed Company shall prosecute its cross-appeal to effect and shall answer all charges and costs that may be awarded against it, if it fails to make its cross-appeal good, then the above obligation is to be void; otherwise in full force and effect.

FUNK BROTHERS SEED COMPANY.
By (signed) Toulmin & Toulmin
Attorneys
THE INDEMNITY INSURANCE COMPANY OF NORTH AMERICA
By (signed) Wallace E. Stafford
Attorney-in-Fact.

(Seal)

Endorsed, Filed,
January 8, 1946,
G. W. Schwaner, Clerk.

1189 And afterwards, to-wit: on the 8th day of February, A. D. 1946, there was filed in the office of the Clerk of said Court in said cause a certain Stipulation which said Stipulation was and is in the words and figures following, to-wit:

1190 IN THE DISTRICT COURT OF THE UNITED STATES
Southern District of Illinois
Southern Division

Kalo Inoculant Company, a corporation, Plaintiff, *vs.* Funk Brothers Seed Company, a corporation, Defendant.	Civil Action No. Q-62

STIPULATION.

It is stipulated and agreed, by and between counsel for the parties in the above-entitled cause, subject to the approval of this Honorable Court, that the time within which the Plaintiff-Appellant and the Defendant-Appellee; Appellant on cross-appeal, may file the record on appeal and docket the appeal in this case pursuant to Rule 73(g) may be and hereby is extended to and inclusive of March 4, 1946.

(signed) Thiess, Olson & Mecklenburger
(signed) M. Hudson Rathburn
Attorneys for Plaintiff
(signed) Toulmin & Toulmin
(signed) H. A. Toulmin, Jr.
(signed) D. C. Staley
Attorneys for Defendant

Approved this 8th day of February, 1946.
(signed) Chas. G. Briggle
United States District Judge
Endorsed, Filed Feb. 8, 1946
G. W. Schwaner, Clerk.

1191 And afterwards, to-wit: on the 11th day of February A. D. 1946, there was filed in the office of the Clerk of said Court in said cause a certain Stipulation which said Stipulation was and is in the words and figures following, to-wit:

1192 IN THE DISTRICT COURT OF THE UNITED STATES
Southern District of Illinois
Southern Division

Kalo Inoculant Company, a corporation, Plaintiff, *vs.* Funk Brothers Seed Company, a corporation, Defendant.	Civil Action No. Q-62

STIPULATION.

It is hereby stipulated and agreed, by and between counsel for the parties in the above-entitled cause, this Honorable Court approving, that the Clerk shall, for the purposes of the appeals now pending herein, transmit to the Circuit Court of Appeals the original exhibits and the original typewritten transcript of the testimony in lieu of copies thereof.

(signed) Thiess, Olson & Mecklenburger
Attorneys for Plaintiff
(signed) Toulmin & Toulmin
Attorney for Defendant

February 11th, 1946.

IT IS SO ORDERED:
(signed) Chas. G. Briggle
United States District Judge

Endorsed, filed,
February 11, 1946.
G. W. Schwaner, Clerk.

1193 And afterwards, to-wit: on the 25th day of February, A. D. 1946, there was filed in the office of the Clerk of said Court in said cause a certain Stipulated Designation of Record on Appeal which said Stipulated Designation of Record on Appeal was and is in the words and figures following to-wit:

1194 IN THE DISTRICT COURT OF THE UNITED STATES
Southern District of Illinois
Southern Division

Kalo Inoculant Company, a corporation, Plaintiff, *vs.* Funk Brothers Seed Company, a corporation, Defendant.	Civil Action No. Q-62

STIPULATED DESIGNATION OF RECORD ON APPEAL.

It is hereby stipulated and agreed, by and between the parties hereto, that for the purposes of the appeal and cross-appeal, heretofore taken in the above-entitled cause, to the United States Circuit Court of Appeals for the Seventh Circuit, that the Transcript of Record to be made up and forwarded to the said Circuit Court of Appeals by the Clerk shall include the following pleadings, papers, documents, testimony and exhibits:

1. Caption. (Usual form)
2. Complaint filed December 10, 1943.
3. Defendant's Answer to Complaint and Defendant's Counterclaim filed January 26, 1944.
4. Plaintiff's Reply to Defendant's Counterclaim filed February 14, 1944.
5. Defendant's Amended Answer to Complaint filed October 28, 1944.
6. Order entered April 30, 1945, setting case for trial at Quincy, Illinois, on May 28, 1945.
7. Order entered May 14, 1945, resetting case for trial at Quincy, Illinois, on June 25, 1945.

1195 8. Stipulation filed June 25, 1945, re use of uncertified copies of patents and publications.

9. Stipulation re claims of Bond patent in suit relied on by plaintiff.
10. The typewritten transcript of the evidence and proceedings including only pages 62 to 824, inclusive.
11. The following original exhibits to be filed with the transcript under Rule 10(5) of the Court of Appeals:

Plaintiff's Exhibits 1 to 7, inclusive, and 10 to 22, inclusive

Defendant's Exhibits 1, 3 to 11, inclusive, 13 to 17, inclusive, and 19 to 36, inclusive

12. Court's Memorandum of Holdings.

13. Findings of Fact and Conclusions of Law, entered October 10, 1945.

14. Judgment entered October 10, 1945.

15. Notice of Appeal by plaintiff filed January 2, 1946.

16. Bond on Plaintiff's Appeal.

17. Notice of Cross Appeal by Defendant, filed January 8, 1946.

18. Bond on Defendant's Cross Appeal.

19. Statement of Points intended to be urged on its appeal by Kalo Inoculant Company, appellant, filed herewith.

20. Statement of Points intended to be urged on its cross appeal by Funk Brothers Seed Company, cross appellant.

21. This stipulated designation.

1196 22. Any further stipulation between the parties hereto concerning the record on these appeals or the time for the docketing thereof and any and all orders of the Court with regard thereto.

(signed) Thiess, Olson & Mecklenburger
Attorneys for Plaintiff

(signed) Toulmin & Toulmin
Attorneys for Defendant

Endorsed, Filed,
February 25, 1946,
G. W. Schwaner, Clerk.

770-771

May 14, 1940. V. S. BOND 2,200,532

BACTERIAL INOCULANT FOR LEGUMINOUS PLANTS

Filed Aug. 24, 1938

Fig 1

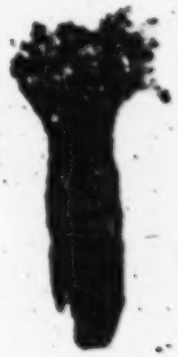


Fig 2

Fig 3

Fig 4

INVENTOR.
Varley Sherman Bond
BY Thiess, Olson & Buckenberger
ATTORNEYS.

PLPS. EX. 2 IDEN.

Patented May 14, 1940 2,200,532

D

UNITED STATES PATENT OFFICE

2,200,532

BACTERIAL INOCULANT FOR LEGUMINOUS PLANTS

Varley Sherman Bond, Quincy, Ill., assignor to Kalo Inoculant Company, Quincy, Ill., a corporation of Illinois

Application August 24, 1938, Serial No. 226,565

14 Claims. (Cl. 71—7)

This invention relates to a bacterial culture and more specifically to a bacterial culture useful for the inoculation of the seeds of leguminous plants.

It is well known that certain plant species of the genus Leguminosae may under certain conditions fix nitrogen directly from the air and convert it to organic nitrogenous compounds. The mechanism of this nitrogen fixation is not understood at the present time, but it has been known for a number of years that it can only be carried out when the plant grows in symbiotic relationship with certain bacteria of the genus Rhizobia. Plants belonging to the genus Leguminosae which are capable of symbiotic relationship with the Rhizobia are peas, beans, alfalfa, red clover, white clover, vetch, lupines, and the like. The bacteria infect the roots of these plants in the soil and cause the formation of so-called nodules, in which it is presumed that the nitrogen fixation in the plant occurs.

Other plants that are important in agriculture, such as the grasses and grains, are not able to fix nitrogen from the air. They depend entirely upon the combined nitrogen in the soil, such as nitrates and ammonium salts, for their nitrogen requirements. Consequently, the combined nitrogen in the soil becomes depleted after a series of crops consisting only of the non-nitrogen fixers have been grown therein. It has consequently become customary in many districts to practice crop rotation, whereby the plants that are capable of fixing nitrogen in the soil will enrich the soil with combined nitrogen, whereby nitrogenous nutrients are provided for a succeeding crop of non-nitrogen fixing plants. More recently it has become the practice to plant mixed crops, which comprise a mixture of the nitrogen fixing and the non-nitrogen fixing variety. Nitrogen is thus supplied to the soil in a combined form which may be readily utilized by the non-nitrogen fixing plants.

Bacteria of the genus Rhizobia which are capable of infecting leguminous plants so that nitrogen may be fixed are widely spread in nature. They are not, however, universally present in soil and it has been found desirable to inoculate the seeds of leguminous plants with a culture of bacteria of the genus Rhizobia previous to the planting of the seeds in the field. This inoculation insures that micro-organisms of the correct species will be present in the soil whereby the leguminous plant may become infected. In the absence of such infection as pointed out above, no nitrogen fixation occurs within the plant. The inoculation of seeds is particularly desirable where it is desired to plant them in a soil that has not grown a plant of the same species for a number of years. In order to insure a maximum amount of nitrogen fixation and development of the leguminous plant, it is always desirable to inoculate the seeds previous to the planting thereof.

Figure 1 shows a root portion of a red clover plant bearing nodules A which were produced by infection with *Rhizobium trifolii*. This view was taken with permission of the authors from a photograph reproduced in the monograph Root Nodule Bacteria and Leguminous Plants, by Fred, Baldwin and McCoy, University of Wisconsin Studies in Science, No. 5, 1932.

Fig. 2 shows a group of forty red clover plants after ten weeks' development in a sterile nitrogen-free sand culture which was not inoculated previous to the time of planting.

Fig. 3 shows a group of forty-one red clover plants of the same age and number grown in the same nitrogen-free substrata as those in Fig. 2, but wherein the seeds were inoculated with a culture of *Rhizobium trifolii* previous to planting.

Fig. 4 is the same as Fig. 3 but wherein the seeds were inoculated with a composite culture prepared in accordance with one embodiment of this invention.

It was discovered early that all species of the genus Rhizobia would not infect all of the plants of the genus Leguminosae to produce nodules wherein the nitrogen fixation presumably takes place. Thus certain species of bacteria are specific to certain species of Leguminosae. The group of plants that will be infected by a single species of bacteria has come to be known in the art as a cross-inoculation group. There are at least six well-recognized species of Rhizobium which will infect six fairly well-defined cross-inoculation groups of plants. *Rhizobium meliloti* will infect alfalfa and white or yellow sweet clovers; *Rhizobium trifolii* will infect red, white, crimson or mammoth clovers; *Rhizobium leguminosarum* will infect garden or field peas, vetch, and broad beans; *Rhizobium phaseoli* will infect garden or field beans; *Rhizobium lupini* will infect the lupines; and *Rhizobium japonicum* will infect the soy beans. The above groups are the most important in regard to inoculation of the seeds of field crops previous to sowing. There is a mixed group, including cow peas, lespedeza, peanuts, velvet beans, lima beans and crotalaria, which has not been well enough defined with re-

spect to cross-inoculation to be assigned a definite terminology. This latter group, however, have been relatively unimportant for the preparation of commercial bacterial inoculants for the seeds of leguminous plants.

A number of so-called commercial cultures of bacteria have appeared on the market from time to time which were purported to be useful for producing nodulation of the roots of the leguminous plants. Many of these cultures have been very effective and useful for the purpose for which they were to be employed. These cultures have appeared in a large variety of forms and contain a large number of viable strains of the desired organism. They have been distributed on suitable jelly bottle slants or in a wet or dry carrying material such as sand, soil, or charcoal.

A variety of methods have also been employed for producing the bacterial inoculant for commercial distribution. In the preparation of an inoculant or culture in accordance with one method, the carrying media, such as humus or charcoal, may be inoculated with a suitable strain of the organism, and sugar, mineral salts, and water may be added with subsequent development of the species of organism therein. The resulting material is then packaged and distributed in any desired manner. This is known as the simple humus culture, but is not entirely satisfactory since it is usually desirable to at least partially sterilize the humus or other carrying material previous to inoculation with the Rhizobia culture in order to insure that the distributed organism will develop within the material to such an extent that a suitable number of organisms would be produced sufficient to inoculate the seeds to be treated. Furthermore, a large amount of metabolic products of the organisms were formed directly in the culture, which do not provide a healthy environment for the organisms, and a large percentage have a tendency to die off in a relatively short time.

A second method that has been employed for the preparation of commercial inoculants has been to develop the organism in a liquid culture and, after the development of the organism in the liquid, mix the liquid with the desired amount of carrying material such as humus or charcoal. This method again has the disadvantage that the metabolic products of the organism and the residual sugars and protein of the culture medium are introduced directly into the commercial product as distributed, which tends to cause deterioration of the culture and the death of a large proportion of the organisms contained therein.

All of the cultures that have been successfully used in the past for the inoculation of the field leguminous plants, such as clover, alfalfa, soy beans, and the like, have contained only one species of the organism. Thus, for inoculation of soy beans, it has been the practice to use a bacterial inoculant comprising substantially a pure culture of *Rhizobium japonicum* in order to obtain maximum plant yield and maximum nitrogen fixation. The same is true with respect to the inoculation of the seeds of the plants belonging to the various other cross-inoculation groups which have been indicated above.

It has heretofore been considered impracticable to prepare a composite culture inoculant containing organisms which will cause nodulation on more than one of the cross-inoculation groups. This has not been done because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen fixation by the plant and the organism was inhibited or even prevented. For this reason it has been considered desirable to use a culture containing a single species of organism rather than one containing a mixture.

The use of a single species of bacteria in a culture presents a serious difficulty in the manufacture and distribution of bacterial inoculants, since it is therefore necessary to distribute a separate culture contained in a separate package for the inoculation of seeds of plants belonging to the various cross-inoculation groups. This situation is further complicated by the fact that the cultures as distributed are ordinarily usable for only a certain length of time after preparation, since the organisms in the culture tend to die out on storage of the culture on the shelves of the distributor. It has consequently become the practice among the distributors of bacterial inoculants of this character to replace cultures free of charge that are unused and over about one year old.

At the present time there are on the market many so-called "pasture mixtures" of seeds which comprise a mixture of seeds including those of different species of leguminous plants. There are distributed in comparatively small quantities, and heretofore the farmer seldom takes the trouble to inoculate the mixture with the several separate bacterial cultures necessary for the seeds of each cross-inoculation group in the pasture mixture.

One object of this invention is to provide a method for the preparation of an inoculant for the seeds of a leguminous plants which will contain a large number of micro-organisms and will retain their viability over a long period of time.

A further object of this invention is to provide a method for introducing a culture of bacteria into a suitable carrying material within which the inoculant is distributed.

A further object of this invention is to provide a mixed culture of Rhizobia capable of inoculating the seeds of plants belonging to several cross-inoculation groups and having the same efficiency in respect to nitrogen fixation as a culture which contains only a single species of the organism.

A further object of this invention is to provide a mixed culture the component strains of which are selected in such a manner that optimum efficiency results when the organisms are combined into a single culture.

A still further object of this invention is the provision of a bacterial culture prepared in admixture with a fine powdered inert material in a new and novel manner.

A further object of this invention is to provide an inoculant for the so-called "pasture mixture" which comprises efficient nitrogen fixing strains of different species of Rhizobia combined in one culture.

Further and additional objects of this invention will appear from the following detailed description, the drawing, and the appended claims.

In order to prepare an inoculant in accordance with one embodiment of this invention, the desired species of organism may be inoculated in any desired manner onto the surface of a suitable agar or jelly culture medium and allowed to grow for a period of several days. A quantity of water may then be added with thorough shaking and further growth permitted, after which time the resulting suspension may be incorporated with a

suitable carrying medium such as humus, charcoal, soil, fine sand, and the like. The bacterial inoculant prepared in accordance with this general method contains an enormous number of organisms which will remain viable over a long period of time. The inoculant is easily handled and may be readily packed in cans or other containers for distribution to the farmer. If the carrying material employed is black humus or charcoal, the inoculant has a further advantage in that it may be readily determined whether or not it has been thoroughly mixed with the seeds previous to inoculation.

In order that this invention may be more readily understood, a specific example is presented in order to more definitely point out the method of preparation of the inoculant. A suitable and preferably pure culture of a bacterial species is selected. It may be obtained by direct isolation from the nodules of a leguminous plant according to methods that are well known in the art. Suitable methods are described by Fred, Baldwin and McCoy, supra, and in the Laboratory Manual of General Microbiology, by Fred and Waksman, 1928. The organism may be cultivated on a yeast extract-sucrose agar which may contain the following ingredients:

Agar	grams	15.0
Sucrose	do	10.0
Dipotassium phosphate	do	0.5
Magnesium sulfate	do	0.2
Sodium chloride	do	0.1
Calcium carbonate	do	1.0
Calcium gluconate	do	1.5
Yeast water (pH 6.8)	cc	50
Tap water	cc	950

The yeast water infusion in the above formula may be prepared as suggested by Fred and Waksman, supra, page 16, as follows: Two kilograms of fresh starch-free pressed yeast are added to 10 liters of tap water. The mixture is steamed for three to four hours at 95° to 100° C. with occasional stirring. The resulting mass is sterilized in an autoclave in deep layers for a period of about one hour. The sterilized material is allowed to stand for about one week, and, if undisturbed, the yeast cells will settle to the bottom and leave a clear straw-colored liquid above. The clear infusion may then be siphoned off and the reaction adjusted to pH 6.8 to 7.0. This clear infusion is the yeast water referred to in the above formula.

The ingredients of the medium are mixed together and sterilized in the usual manner. The calcium carbonate may be sterilized separately and added to the sterile medium after it has cooled down in order to prevent the decomposition of sucrose during the heating period. The medium prepared in accordance with the formula given above is a jelly on the surface of which cultures of the Rhizobia may readily develop under aerobic conditions. It may be also prepared in the liquid form by eliminating the agar.

The desired culture of the Rhizobia may be inoculated onto an agar slant of the medium and incubated at suitable temperatures for a time to permit development of the culture on the surface of the agar. This first transfer of the culture is generally made in test tubes. After a suitable period of incubation, the culture may then be transferred by a sterile needle or loop from the test tubes to an agar slant in a 200 cc. flat bottle containing about 30 cc. of slanted agar media. Here the organism is again permitted to develop for a suitable incubation period at 28° to 30° C., which in most cases will extend from a few days to over a week. The culture grown on the agar surface of the bottle may then be aseptically washed from the surface with sterile water according to recognised bacteriological technique whereby a suspension of the organisms is produced. This suspension, being quite heavy and almost opaque, may be used for the inoculation of slanted agar media contained in a plurality of still larger bottles such as one liter bottles. These bottles may contain about 125 cc. of the agar medium described above. The inoculation may be made by streaking with a needle or aseptically spraying a water suspension of the organisms onto the surface of the agar by an atomizer or any suitable spraying means. It is desirable that aseptic conditions be maintained throughout the process until after the large bottles have been inoculated with a pure culture of the organism. Prevention of contamination up to this point is desirable, since the contaminating organism may outgrow the desired species of the Rhizobia, thus negativing the effect of the culture for seed inoculation purposes.

After the culture has developed on the surface of the agar in the large bottles for a suitable length of time (from about 2 to 10 days), tap water may be added in order to fill the bottles about half full. They are thoroughly agitated and the agar is cut or broken with a suitable metal probe, whereby the bacteria are rinsed from the surface of the agar and the agar becomes thoroughly broken up in the bottom of the bottle. It may be desirable, but it is not necessary, to employ sterile water for this purpose. The growth of the Rhizobia has taken place to such an extent that any chance contaminant will readily be suppressed by the large numbers of the Rhizobia.

After the agar has been thoroughly broken up, the large bottles are allowed to further incubate at suitable temperatures for a period of a few days. It has been found that the number of the organisms becomes very greatly increased during this subsequent period of incubation. The agar may then be separated from the suspension of organisms by passing the suspension through a suitable filter or screen. A four mesh screen has been found to be satisfactory. The bottles and agar may be washed with a small quantity of tap water. The resulting filtered suspension of bacteria is very heavy, being milk-like in appearance. This suspension may then be thoroughly incorporated with any desired amount of humus, which serves as a carrying means for the organism of the suspension. The humus and suspension mixture is allowed to stand for about 12 to 24 hours before the material is packed in order to permit complete absorption of the moisture by the humus. The resulting composition may then be packaged in moisture-proof fiber cans or other suitable containers for shipment.

The material from seventy-two one liter bottles inoculated and treated as described above is sufficient to inoculate about ninety kilograms of humus in order that the concentration of organisms in the humus will be approximately sixty million per gram. The final product may contain about 45% moisture. The water content may be adjusted by adding water in the desired proportions previous to packaging.

It will be understood, of course, that other types of media may be employed for the development of the organism preliminary to its inoculation into the humus or other carrying material, and the proportions of the various ingredients may be varied within a wide latitude. Likewise, charcoal,

fine sand, soil, kieselguhr, and other materials may also be used as the carrying medium. If humus is employed, it may be desirable to neutralize its natural acidity with hydrated lime or calcium carbonate. It is not necessary, as has been previously stated, that sterile conditions be maintained after the incubation period on the surface of the agar immediately preliminary to the addition of the excess water. Sterile conditions are not necessary after this point, since the organisms have increased to such large numbers that they will suppress any type of contaminating organism that may be introduced. Likewise, it is not necessary to sterilize the humus or other carrying material because of the tremendous number of organisms of the desired type that are introduced therein. It will be readily recognized, however, that, if desired, strict aseptic conditions may be maintained up until the time the material is packaged in the containers for shipment.

The culture prepared in accordance with this method contains a sufficient number of organisms so that 150 grams will inoculate about one bushel of small seed.

It will be understood from the above description that the number of organisms incorporated into the humus may be readily predetermined by means of a Petroff-Hausser bacterial counter or other suitable direct counting apparatus. This method, therefore, has the advantage that the number of organisms being introduced into the final inoculant may be readily determined. The final culture is also comparatively free of excess sugar and minerals, since they are retained to a large extent by the agar in the large bottles. Consequently, additional nutrient is not introduced into the humus which may stimulate the development of contaminating organisms.

It will be understood also that the incubation period at the different stages of preparation of the culture previous to inoculating into the carrying means depends on many factors recognized by one skilled in the art. Thus, the character of the media, the temperature of incubation, and the species of Rhizobium are factors to be considered.

In order to inoculate the seeds of leguminous plants by the inoculant prepared in accordance with the above given description, the seed, within about twenty-four hours previous to the time of planting, is slightly moistened and a predetermined quantity of the inoculant is thoroughly mixed therewith in order to provide the desired concentration of organisms on the seed. The distribution of the inoculant throughout the seeds may be readily observed because of the black color of the humus with which the inoculant is prepared. The inoculant prepared in accordance with this invention may be satisfactorily used for a period of more than one year and sufficient organisms remain viable during this time in order to provide a number satisfactory for thorough inoculation.

As has been pointed out above, composite cultures for inoculating the seeds of leguminous plants have not generally come into use because of the inhibitory effect that certain strains of certain species of the Rhizobia have on each other and also because no method has been developed whereby a composite culture may be produced wherein a sufficient number of each species of organism in the inoculant is insured.

In order to more clearly point out the nature and magnitude of the inhibitory effect of several species of Rhizobia, tests were carried out using strains of various organisms which were picked at random and unselected in the manner to be hereinafter described. Thus a culture of *Rhizobium japonicum* which was known to be an efficient culture for fixing nitrogen when in association with the soybean plant was tested in order to determine its nitrogen fixing efficiency when inoculated onto soybean seeds in the presence of *Rhizobium trifolii* and *Rhizobium meliloti*. The tests were made by growing the appropriate plants in sterilized nitrogen-free sand according to well known methods in the art and fully set forth by Fred and Waksman, supra, and Fred, Baldwin and McCoy, supra. Three groups of soybean plants containing eight plants in each group were planted in a nitrogen-free sand containing suitable mineral salts and according to the standard methods. One group of seeds were planted sterile, being uninoculated. The seeds of the second group were inoculated with a single strain of the species of *Rhizobium japonicum* indicated above. The third group of seeds was inoculated with a mixture of the same strain of *Rhizobium japonicum* and strains of *Rhizobium trifolii* and *Rhizobium meliloti*. The seeds were permitted to germinate and the plants were grown in the sand for a period of fifty-six days. The eight plants developing from each group were then analyzed for total nitrogen by a suitable Kjeldahl procedure. The plants of the first group (uninoculated) contained a total of 79 milligrams of nitrogen. The plants of the second group (single strain) contained 246 milligrams of nitrogen. The plants of the third group (same strain mixed with strains of other species) contained only 203 milligrams of nitrogen. Consequently the plants of the second group fixed 167 milligrams of nitrogen over the control, and the plants of the third group only fixed 124 milligrams of nitrogen over the control. Thus it will be seen that this strain of *Rhizobium japonicum* produced 34.8% more nitrogen when employed alone than when employed in the presence of strains of other species of Rhizobia.

In the same manner a strain of *Rhizobium trifolii* which efficiently fixed nitrogen in the presence of the host plant alone was tested. Groups of forty plants were inoculated in this instance in the manner indicated above which is well recognized in the art. It was found that this unselected strain of *Rhizobium trifolii* caused about 118% more nitrogen to be fixed in the host plant when employed alone as an inoculant than when in the presence of other species of the Rhizobia, such as *Rhizobium japonicum* and *Rhizobium meliloti*.

It is possible, in accordance with one embodiment of this invention, to produce an effective composite inoculant containing a plurality of species of organisms which is capable of infecting a variety of leguminous plants belonging to a plurality of cross-inoculation groups.

In general, the composite culture may be prepared by selecting strains of the desired species of the Rhizobia which will produce optimum beneficial results to the host plant and will not be adversely affected by each other. The strains, after having been selected, may be cultivated in a manner which has been described above on agar or jelly media separately, the heavy suspension of each organism being introduced directly into the humus or other carrying material in the manner that has been previously described.

In order to produce a composite inoculant

containing, for example, *Rhizobium meliloti*, *Rhizobium trifolii*, and *Rhizobium japonicum*, which inoculant will infect the corresponding cross-inoculation groups, a number of strains of each of these organisms may be isolated from nature by any of the approved methods such as have been described in the above referred to Laboratory Manual of General Microbiology by Fred and Waksman. Each of the strains are tested separately with its own host plant in order to select out those which are most effective in respect to the development of the host plant and the quantity of nitrogen fixed thereby. These tests may be made according to standard practice by aseptically growing the plants in a nitrogen-free substrata such as sand containing mineral salts. The cultures showing the best results in association with the host plant may then be selected and the various strains of the different species may be mixed together in order to determine if the nitrogen-fixing ability of one species is adversely affected by the mere presence of another, as has been previously thought to be the case. Thus, it has been previously considered that the nitrogen-fixing ability of *Rhizobium trifolii* in symbiotic relationship with red clover is markedly inhibited if strains of *Rhizobium meliloti*, *Rhizobium japonicum*, or other species of the Rhizobia were present within the same inoculant. It has been found, however, that, by a suitable process of selection of strains in the manner indicated above, a composite culture may be obtained which may be used to infect a plurality of cross-inoculation groups with optimum results.

The organisms of the genus Rhizobium which are mutually noninhibitive may be designated by the term alpha. No method has been observed whereby the alpha strains of the organisms may be differentiated from the mutually inhibitive group of Rhizobia other than by making actual nitrogen fixation tests by inoculating the various host plants with the proposed mixture of the Rhizobia. A composite culture that is satisfactory for use for inoculating seeds of alfalfa, red clover, and/or soy beans (representatives of three cross-inoculation groups) contains a mixture of *Rhizobium meliloti alpha*, *Rhizobium trifolii alpha*, and *Rhizobium japonicum alpha*. All of the strains may be selected so as to be mutually noninhibitive when mixed and used for seed inoculation. It will, of course, be recognized that certain inhibitive strains may be employed in the mixture. In general, however, it is desirable to use the alpha strains which have been selected in the manner proposed in the above. By the alpha strains, which have been designated herein, is meant those strains of the species of Rhizobia which are mutually noninhibitive of each other as regards nitrogen fixation in the host plant. It is understood that the alpha strains of each species may vary somewhat from each other in minor physiological, morphological and/or immunological characteristics. All such variants may be included within the scope of the term alpha strain.

One criterion by which the alpha strains of the organisms may be determined is based on a statistical comparison of the total milligrams of nitrogen fixed in plants previously inoculated with a single strain unmixed with strains of other species with the total milligrams fixed in plants inoculated with the same strain mixed with strains of other species. In making such tests the conditions for inoculation and growth of the plants should be kept uniform when testing both the mixed culture and the single strain. The method for carrying out tests for determining the amount of nitrogen fixed by leguminous plants in association with the various species of the Rhizobia are well known in the art and are given in detail by Fred and Waksman, supra, and Fred, Baldwin and McCoy, supra.

Each culture may be tested by inoculating a given number of seeds of the proper host plant with a strain of culture of bacteria to be tested either singly or mixed with other species of the Rhizobia. The seeds may then be planted in jars containing sterile sand and appropriate nitrogen-free mineral salt solutions capable of supporting plant growth. The same number of seeds are planted in each of five jars for each culture being tested, and the total number of milligrams of nitrogen fixed by the plants in each of the five jars is tested. The standard deviation of the mean amount of nitrogen fixed in each jar may then be determined by appropriate statistical methods. If the mean plus or minus the standard deviation of the nitrogen content of plants inoculated with a strain used unmixed with the strains of another species, overlaps the mean plus or minus the standard deviation of plants inoculated with the same strain used mixed with other species of Rhizobia, that strain may be considered as nonantagonistic or an alpha strain within the meaning of this invention.

For the preparation of a composite bacterial inoculant in accordance with this invention, heavy suspensions of selected cultures of *Rhizobium trifolii alpha*, *Rhizobium meliloti alpha*, and *Rhizobium japonicum alpha* may be prepared in the same manner as has been indicated above for the preparation of a single culture in accordance with this invention. The heavy suspensions may then be mixed and introduced into the humus in concentrations suitable to give the desired results.

In order that 90 kilograms of dry humus may be properly inoculated with the three organisms indicated above, about 19 liters are prepared of each suspension in the manner previously indicated. The concentration of organisms in the suspension may be about 900 million per cubic centimeter for *Rhizobium meliloti alpha* and *Rhizobium trifolii alpha* and about 450 million per cubic centimeter for *Rhizobium japonicum alpha*. The suspensions may be introduced into the humus or other carrying material separately or may be mixed before introduction. The humus, after inoculation with the three organisms (about 57 liters of composite suspension), may be passed through a suitable mechanical mixer in order to thoroughly incorporate the organisms within the humus or carrying material. The water content of the humus may be brought up to about 45% and the mass is permitted to stand for about 12 to 24 hours preliminary to packaging in order to permit a more thorough absorption of the water and bacterial cultures into the humus. This latter storage period also causes the humus to become lighter and more easily handled by the packing machines, which may be of any desired kind. One hundred seventy-five grams of this inoculant is sufficient to inoculate one bushel of red clover or alfalfa seed or two bushels of soy bean seed.

The resulting composite inoculant may be used for inoculating the seeds of leguminous plants in the same manner as hereinbefore set forth. It

may be used with good results for inoculating the seeds of any of the plants included within the cross-inoculation groups represented by the bacterial species present. The particular example given has included *Rhizobium trifolii alpha*, since it has been generally thought that *Rhizobium trifolii* was particularly sensitive to other of the Rhizobia in respect to its ability to fix nitrogen within the host plant. It has been found, however, that, by suitable selecting, strains of the organism may be found from which a composite inoculant may be produced having all of the desirable qualities of a single strain culture with the further additional advantages previously indicated.

It will be understood that this invention is not to be limited to the above described method for producing a composite inoculant. It is not necessary that it be prepared as set forth in the manner described above. Any inoculant comprising a mixture of selected anti-inhibitive strains of ordinarily inhibitive organisms of the Rhizobia group is included within the spirit and scope of this invention.

In order that a more definite understanding may be had of the advantages to be derived from the use of a composite culture selected and prepared in accordance with this invention, a series of tests were made by planting red clover seeds in half gallon jars containing sterile sand supplemented with all essential minerals except nitrogen, and were inoculated as indicated below. After ten weeks of growth in the greenhouse under identical conditions, the sand was washed from the roots of the plants, the plants dried, weighed and ground and samples were analyzed by the Kjeldahl method for their nitrogen content.

Each group or series of seeds was planted in a series of five jars and the standard deviation between the jars of each culture series was determined. Each series was inoculated as follows:

1. Uninoculated control plants.

2. Plants inoculated with a non-selected strain of *Rhizobium trifolii* in single culture unmixed with other species.

3. Plants inoculated with a selected, non-antagonistic strain of *Rhizobium trifolii alpha* in single culture unmixed with other species.

4. Plants inoculated with same organism as series 2 in admixture with other species of the Rhizobia.

5. Plants inoculated with the same organism as series 3 in admixture with other species of Rhizobia.

The following table is presented which indicates the inoculant used for each series of plants, the dry weight of ten plants, the per cent of nitrogen in the dry material, the milligrams of nitrogen present per ten plants, and the milligrams of nitrogen fixed per ten plants:

Inoculant series No.	Grams dry weight per 10 plants	Percent nitrogen	Mg. nitrogen per 10 plants	Mg. nitrogen fixed per 10 plants
1	0.61	1.07	6.5± 0.5*	
2	4.12	2.92	122.0± 10.5*	115.5± 10.9*
3	4.60	2.85	131.0± 16.9*	124.5± 15.5*
4	3.83	2.39	89.5± 4.7*	72.5± 3.9*
5	4.80	2.82	135.0± 40.5*	119.5± 39.7*

*Standard deviation of the mean of a series of 5 jars.

It will be observed that the nitrogen fixing efficiency of the inoculants of series 2, 3 and 5 are high and substantially the same. When the non-selected strain, however, was mixed with other species of Rhizobia the amount of nitrogen fixed by the host plant was seriously impaired as indicated by series 4. This example clearly shows the increase in effectiveness of a mixed culture which contains an alpha strain over that which contains a non-selected but otherwise efficiently nitrogen fixing strain. It is pointed out here that the mean plus or minus the standard deviation of the nitrogen fixed by series 4 does not fall in the same range as the mean plus or minus the standard deviation of that fixed by series 2. Series 3 and 5 do overlap in this respect and the strain of *Rhizobium trifolii* employed in those tests was consequently an alpha strain according to the one criterion that has been previously described.

It is to be understood that this invention is not to be limited to the selection of a single strain which is to be used in the composite culture. All of the strains may be selected in the manner indicated previous to the preparation of the inoculant. Furthermore, if desired, a plurality of strains of the same species may be introduced into the composite culture containing several species. This practice has been found to be desirable in many instances in order that there will be double insurance that an effective nitrogen fixing strain of the specific organism is present within the culture.

Referring more particularly to the drawing,

Fig. 1 is a photolithograph picture of a root system of a red clover plant having nodules A located near the crown of the root. Large nodules on the plant near the crown are indicative of good nitrogen fixation and a good strain of the organism.

Fig. 2 shows a control group of forty red clover plants which have grown in a nitrogen-free sand culture for ten weeks with no Rhizobia present. This group had a wet weight of 20.4 grams and a total nitrogen content of 27 milligrams derived from the original seeds. No nitrogen was fixed.

Fig. 3 shows the forty-one plants grown under identical conditions as those shown in Fig. 2 but which were inoculated with a humus inoculant containing only bacteria of the species *Rhizobium trifolii alpha* prepared in accordance with this invention. The wet weight of this group of plants was 156.7 grams and had a total nitrogen content of 645 milligrams of nitrogen. Thus 618 milligrams of nitrogen were fixed.

Fig. 4 shows a group of forty plants the same as Fig. 3 which were inoculated with a composite inoculant prepared in accordance with one embodiment of this invention containing strains of the species *Rhizobium trifolii alpha*, *Rhizobium meliloti alpha*, and *Rhizobium japonicum alpha*. It will be noted that the red clover plants inoculated with the composite inoculant containing the alpha strains are fully developed to the same extent as the one inoculated with the pure culture and no inhibition is observed in the plant development because of the presence of the other organisms. The wet weight of this group was 159.0 grams and had a total nitrogen content of 662 milligrams. Thus 635 milligrams of nitrogen were fixed.

It will be observed that the organisms of the composite culture described in the foregoing example may be introduced into the culture in any desired numbers and the relative proportion may be fairly accurately predetermined by means of direct bacterial count. The cultures are not mixed until immediately preliminary to their introduction into the carrying material and after

they have developed to substantially their maximum numbers. Consequently, there is no danger of one species overgrowing the other during the process of incubation.

The invention is not to be limited in any way to the number of organisms contained within the inoculant or the number necessary for inoculating a predetermined quantity of seed. In practice, and as shown herein, a large excess of bacteria is generally introduced into the carrying material in order to insure that all of the seeds are thoroughly inoculated with a sufficient number of organisms to produce the desired results.

While several particular embodiments of this invention are shown above, it will be understood, of course, that the invention is not to be limited thereto since many modifications may be made, and it is contemplated, therefore, by the appended claims to cover any such modifications as fall within the true spirit and scope of this invention.

I claim:

1. An inoculant for leguminous plants comprising a plurality of selected cultures of different species of bacteria of the genus Rhizobium, one of said cultures being *Rhizobium trifolii alpha*, said cultures being substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

2. A bacterial inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium comprising *Rhizobium trifolii alpha*, *Rhizobium meliloti alpha*, and *Rhizobium japonicum alpha*.

3. An inoculant for leguminous plants comprising humus and a mixture of species of bacteria of the genus Rhizobium, said mixture containing a strain of *Rhizobium trifolii alpha*.

4. An inoculant for leguminous plants comprising a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus Rhizobium, said strains being unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

5. An inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium, at least one of which strains is unaffected by the others in respect to its ability to fix nitrogen in the leguminous plant for which it is specific.

6. An inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium, the bacteria of each strain being present in numbers of substantially the same order of magnitude, said strains being substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

7. A bacterial inoculant for leguminous plants comprising a moist powder base and a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus Rhizobium including *Rhizobium trifolii*, the bacteria of each strain being present in numbers of substantially the same order of magnitude, said strains being substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

8. A bacterial inoculant for leguminous plants comprising a moist powder base containing a substantially negligible concentration of bacterial nutrients and a plurality of strains of different species of bacteria of the genus Rhizobium, at least one of which strains is unaffected by the others in respect to its ability to fix nitrogen in the leguminous plant for which it is specific.

9. A method for producing a bacterial inoculant for leguminous plants which comprises separately selecting strains of different species of the genus Rhizobium, separately cultivating said strains in nutrient media, and mixing the resulting cultures whereby a composite inoculant is produced, said strains originally being selected so that they are substantially non-inhibitive and non-antagonistic to each other in respect to their ability to fix nitrogen in the host plant for which they are specific.

10. A method for producing a composite inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium, which comprises cultivating each strain separately in suitable culture medium to produce a substantial quantity of living organisms and thereafter mixing the organisms with a moist powder base containing a substantially negligible concentration of bacterial nutrients whereby the desired inoculant is formed.

11. A method for producing a composite bacterial inoculant for leguminous plants which comprises separately cultivating a plurality of strains of bacteria selected from different species of the genus Rhizobium and subsequently mixing the resulting cultures containing large numbers of bacteria with a moist powder base containing a substantially negligible concentration of bacterial nutrients whereby the desired inoculant is obtained, the bacteria of each strain being present in numbers of substantially the same order of magnitude.

12. A method for producing a composite inoculant for leguminous plants which comprises separately cultivating a plurality of species of bacteria of the genus Rhizobium in separate cultures and mixing with a powder base predetermined numbers of the bacteria of each culture to produce the desired inoculant, said powder base being substantially free of added bacterial nutrients.

13. A bacterial inoculant for leguminous plants comprising a plurality of strains of different species of bacteria of the genus Rhizobium in numbers of substantially the same order of magnitude incorporated with a powder base, the mixture being substantially free of bacterial nutrients whereby growth of the bacteria therein is inhibited.

14. The inoculant as defined in claim 13 wherein one of said species is *Rhizobium trifolii*.

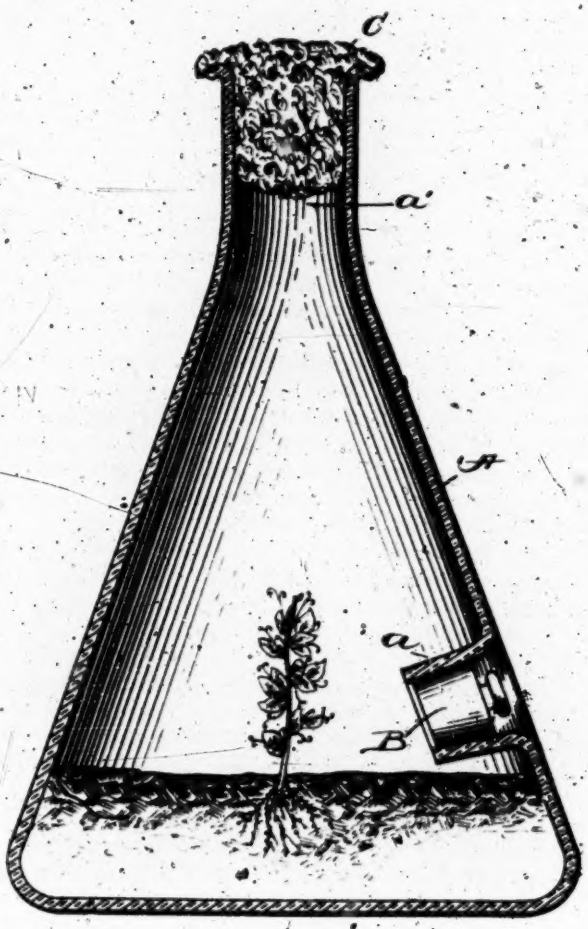
VARLEY SHERMAN BOND.

No. 865,965. PATENTED SEPT. 10, 1907. 780-78

G. H. EARP-THOMAS.

ART OF GROWING AND DISTRIBUTING NITRO GATHERING BACTERIA.

APPLICATION FILED JAN. 17, 1907.



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DEFTS. EX. 20 IDEN.

UNITED STATES PATENT OFFICE. 782-783

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ART OF GROWING AND DISTRIBUTING NITRO-GATHERING BACTERIA.

No. 865,965. Specification of Letters Patent. Patented Sept. 10, 1907.

Application filed January 17, 1907. Serial No. 352,768.

To all whom it may concern:

Be it known that I, GEORGE HERBERT EARP-THOMAS, a citizen of New Zealand, residing at 400 Bloor street, Toronto, Canada, have invented certain new and useful Improvements in the Art of Growing and Distributing Nitrogen-Gathering Bacteria; and I do hereby declare the following to be a full, clear, and exact description of the invention, such as will enable others skilled in the art to which it appertains to make and use the same.

My invention relates to improvements in the art of growing and distributing nitrogen gathering bacteria.

It is known that certain bacteria which live at the roots of plants have the power of collecting nitrogen from the air and supplying it to the said plants. It is also known that it is advantageous to grow cultures of such nitrogen gathering bacteria in laboratories and distribute the same to agriculturists in order that they may directly inoculate the seeds before planting, whereby an increased growth of the plants and an increased yield is obtained. In growing such cultures in the laboratory it has been the practice heretofore first to obtain some of the desired bacteria from plants taken from the field or garden and then to implant such bacteria so obtained upon a suitable medium which is then kept under the desired conditions to propagate the colonies, the culture thus obtained being generally known as the stock culture, which laboratory stock culture serves as a source of germs from which to propagate the cultures to be distributed to the agriculturists. Unfortunately, the process of obtaining a satisfactorily pure culture direct from plants growing in the field or garden is a tedious and slow procedure, because of the difficulty of excluding contaminations, such as molds, yeasts and soil bacteria of a kind other than that desired. It might seem that once a pure culture of the desired bacteria had been obtained that there would never be any further need to go again to the growing plant for the germs, but that from the stock culture other cultures might be propagated indefinitely. However, owing to the fact that the characteristic properties of these bacteria become modified by the food upon which they live, it results that a laboratory stock culture, after some time, consists of bacteria which have, to a certain extent, lost their power of fixing nitrogen for the plant, or in other words have become what is known as attenuated, that is to say, less virulent than before and therefore less valuable to the farmer for inoculating seed than a freshly prepared stock culture. Therefore, under the present methods, it is customary to discard the stock culture from time to time and make a fresh one from the virile bacteria of the soil grown living plant.

It is one of the objects of my invention to avoid the above described disadvantage, and this I do by the means more fully explained hereinafter.

There is also a further great disadvantage connected with the old method, which is due principally to the necessity for the frequent renewal of the stock culture and to the great similarity which exists between the nitrogen gathering bacteria and other soil bacteria which are useless for inoculating seed. This disadvantage is that when a new culture has been made, if it be examined under the microscope, it may appear to be the desired nitrogen gathering bacteria and yet really may be some form of soil bacteria useless for the purpose required. Apparently the only really reliable test of a culture is to put it in practical use, that is, to inoculate seed with it and then grow a plant from such seed. This test has been employed heretofore, but under such conditions as often to render the test inconclusive, for the reason that the possibility of the inoculation of the plant from sources other than the stock culture, was not absolutely excluded. Consequently, by the old method of procedure it is possible to have cultures of bacteria satisfactorily pass all the tests to which they have been submitted and be sent to the farmer as nitrogen-gathering bacteria, when in fact they were not nitrogen gathering bacteria but were merely some other form of bacteria which were useless for the purpose intended.

It is a further object of my invention to avoid this disadvantage, which I do by the means hereinafter explained.

Furthermore, although the nitrogen gathering bacteria so far generally known are supposed to be and probably are of one species only, the results attained in actual practice have shown that there is a difference in the bacteria dependent upon the species of plant upon which the stock culture was obtained. For example, bacteria obtained originally from the roots of red clover will not produce as satisfactory results when employed for inoculating alsike as would bacteria originally obtained from the roots of an alsike plant, and on the other hand the latter will not be satisfactory for the inoculation of red clover. Apparently the characteristics of the bacteria are modified by the food upon which they live, so that when for example, the red clover bacteria are placed upon red clover seed they are ready to thrive on the roots of the growing plant, whereas when placed upon some other seed, as for example, on alsike seed, they find their environment and food less suitable to them and hence are not as vigorous in their attacks on the plant roots. However, the bacteria have the power of finally adapting themselves to the changed conditions, that is to say, after many generations are produced upon the new kind of host the race finally becomes accustomed to the new host and is enabled to carry on its function satisfactorily. Unfortunately, this adaptation of the bacteria to its strange host requires some time, and the consequence is that seed inoculated

with bacteria unadapted to it does not have the advantage of a quick growth when first starting and thus a part of the growing season is lost, which would not be the case were the proper kind or variety of bacteria present on the seed at the moment of germination. Owing to this necessity for employing the proper kind of bacteria for each kind of plant, it has been necessary to maintain many different kinds of stock cultures in the laboratory each containing bacteria adapted for a particular plant. Also it has been necessary first to ascertain from a purchaser, the kind of plant which he intended to inoculate before his order could be filled. And as it is necessary to make up the cultures in distributing packages prior to the opening of the season for which they are to be used, so that orders may be filled without delay, it has been necessary to make a supply of distributing packages of each kind of bacteria.

It is a further object of my invention to avoid this difficulty which I do by the means hereinafter described.

With these general objects in view, and some others which will be obvious to those skilled in the art from the description hereinafter, my invention consists in the features and details which will first be described in connection with the accompanying drawing and then particularly pointed out in the claims.

The drawing is an elevation of a stock culture embodying one part of my invention.

Referring to the drawing, A is a receptacle or container, in this case of suitable transparent material, as for example, glass, and provided with an opening at one side arranged to be closed by a suitable closure. In the present example, the desired result is attained by providing the receptacle with a tubulature indicated at *a* which may be closed by a stopper which will exclude germs, as for example, a tightly fitting glass stopper B.

The upper part of the receptacle A is provided with means for supplying it with sterilized air that is to say air which is free from germs of any kind. In the present instance, I obtain this result in a simple manner by providing the receptacle A at the upper part with an opening, *a'* in which may be inserted a filtering device for filtering the air so that all germs will be removed as the outside air passes through the filter into the receptacle A. As a filter a plug of cotton is generally employed, this being indicated at C.

Within the receptacle, I place a suitable medium, taking care to have both sterilized by any well known methods, the medium in the preferred form of my invention being non-nitrogenous and substantially transparent. In my Patent 816,850, dated April 3, 1906, I have described a medium which is very suitable for this purpose. In this medium, I place one or more seeds taken from the desired kind of plant in such a way as to insure that the seed is free from bacteria of any kind. For the purpose of accomplishing this result I may proceed in the following manner. I take a seed-head of the desired plant and carefully sterilize the same by passing it several times through the flame, such as the flame of an alcohol lamp, in order to make certain that all organisms on the outside of the seed-head are destroyed by the heat. The seeds themselves within their seed cases or pods are not injured by the heat if the work is properly done. Apparently there are no injurious germs within the seed-pod, if the latter is perfect, that is, if it has not been punctured, whereby it results that the sterilization of the exterior of the seed-head in a manner such as described will enable the operator to remove the seeds from their pods by means of suitable sterilized instruments and transfer the same under the usual aseptic laboratory conditions, to the medium in the receptacle. Thereafter, the medium may be inoculated with the desired culture of nitrogen gathering bacteria, through the tubulature. The receptacle thus prepared may be placed in a suitable situation in order to receive a proper amount of light and heat, whereby the seed germinates and the bacteria at once start to work in the rootlets.

The medium being non-nitrogenous, the bacteria are compelled to obtain their entire supply of nitrogen from the atmosphere. The plant in turn takes its nitrogen from the bacteria and thus grows rapidly, a little sterilized water being added to the medium from time to time as may be required. As the bacteria are growing and multiplying upon their natural host, they have a vitality and obtain a virulency which is even greater than that of the bacteria living upon a plant growing under natural conditions in earth. Furthermore, as the medium is transparent the development of the colonies may be watched and the growth of the plant itself serves to determine whether the bacteria in the medium are the true nitrogen gathering bacteria or not for if they are not the plant will not thrive, whereas if they are the plant will flourish and will develop tubercles on the roots. Such a laboratory stock culture being once established there will be no need thereafter to go to the original source for bacteria, namely, to the soil grown plant, because there is not only no loss in virulency of the bacteria on a stock culture such as I have described, but if anything an increase above the average of those cultures made directly from the soil-grown plant. When now it is desired to make other cultures from the described stock culture it is only necessary to remove the stopper from the tubulature, insert a sterilized pair of forceps and remove a tubercle from the roots of the plant which may then be used in any desired manner to inoculate the new medium. The bacteria thus obtained are in a most virulent form and absolutely reliable. The danger of contamination is substantially eliminated and the new cultures are produced quickly and with certainty.

Although I have referred above to the use of the tubercle for inoculating a fresh medium from the stock culture, it is also possible to use some other portion of the root of the plant, as for example, a piece of one of the small root hairs, for these also contain some bacteria.

While I have hereinabove described the receptacle and its contents as a stock culture, it is also to be understood that when a stock culture is obtained by the old method, my invention may be employed as a means for testing such stock culture. That is, the seed may be planted in the medium in the receptacle

just as hereinbefore described and then inoculated not to serve as a stock culture, but merely to determine whether or not the bacteria employed are of the proper kind. For such a purpose the receptacle may be smaller, because it is not necessary to provide such room for the plant. The first growth of the plant will finish the test and determine whether the bacteria are of the desired kind or not. In preparing these test cultures, it is advisable to make the medium as transparent as possible and the receptacle may be small enough so that it can conveniently be examined under the microscope. I have found an ordinary test tube with a plug of cotton in its open end to serve satisfactorily. With such test cultures it is possible to observe the bacteria at work upon the plant. This test is particularly useful in exposing to view the nitrogen gatherers which enter the roots but for some reason do not form nodules. These bacteria are often sufficiently energetic and numerous to aid the plant without any external evidence of their presence being visible.

In order to be prepared to fill all orders which may be received for cultures to inoculate the various kinds of plants which it is customary to inoculate, a series of stock cultures each with a different species of plant growing therein may be prepared and kept on hand. The distributing vessels may be prepared and filled with medium, inoculated and sealed in the manner described in my said Patent 816,850 each distributing vessel being inoculated with its own kind of bacteria from the desired stock culture. However, I have found that it is an important improvement upon the process set forth in said patent to proceed as follows: After the distributing vessel has been sterilized, supplied with a semi-solid or rather substantially solid sterilized medium, inoculated and incubated in the manner described in my said patent, a small portion of sterilized water should be poured on top of the medium and the receptacle then closed, as for example, by hermetically sealing the same; whereupon the distributing package is ready for shipment or storage.

The advantage of the solid medium is that there is less danger of spilling the same in handling the vessels prior to sealing and hence less care is required in placing them in the incubator. But the foremost advantage is that the growth of the colonies may be observed with a solid medium whereas it cannot with a liquid medium. The advantage of the liquid medium is that the bacteria can subdivide more readily than in a semi-solid medium, and hence will propagate quicker. Therefore, by following the process set forth above, I attain the advantage of a solid medium during the period of preparation of the cultures prior to sealing and yet also attain the advantage of the liquid medium after sealing, thus leaving the bacteria in a favorable condition for a further development during storage and shipment.

In the above description, I have assumed that the medium in the distributing vessels is inoculated with one kind only of the nitrogen gathering bacteria. However, an important feature of my invention consists in inoculating the medium in each distributing vessel with a plurality of kinds of nitrogen gathering bacteria, as will now be more fully explained. To do this, I first form a composite colony of a plurality of kinds of bacteria, by inoculating some medium in a suitable vessel with cultures taken separately from the different stock cultures, thus combining in one culture bacteria suitable for different kinds of plants. After this composite stock culture has been formed, the distributing vessels are inoculated from it whereby instead of each distributing vessel containing only one kind or variety of the nitrogen gathering bacteria, it contains a plurality of kinds. Consequently, such a culture is suitable for use in inoculating the seed of anyone of the kinds of plant from which the bacteria were originally obtained. However, and this is an important feature of my invention, it is not necessary to use as much if any larger quantity of such a composite culture in inoculating the seed than is required with the simple culture, for the reason that there is enough of the specific kind of bacteria to attack the rootlets at the time the seed germinates and thus start the young plant into rapid growth by which time the other kinds of bacteria have adapted themselves to their changed conditions and then can thrive on the plant roots and assist in the growth of such plant. A further advantage of this plan is that it gives a means of graduating the intensity of the attack on the young rootlets of the germinating seed while at the same time, insuring the presence of sufficient bacteria to properly stimulate the growth of the plant after it has arrived at a more vigorous stage.

From laboratory experiments, I have ascertained that it is possible to injure instead of aid the germinating plant by the employment of too large a colony of virulent bacteria during the germinating of the seed. In fact, in experiments carried out by me with peas, the bacteria had not only retarded the growth of the rootlets but had actually reduced the pea seed to a mere husk or shell. All danger from this source is entirely avoided by the use of the composite culture.

Having thus described my invention, what I claim as new and desire to secure by Letters Patent, is:

1. The process of developing a bacteriological culture, which consists in growing in a medium a plant inoculated with the desired bacteria while excluding all contaminations from said plant and medium.

2. The process of developing a bacteriological culture, which consists in growing in a non-nitrogenous medium, a plant inoculated with nitrogen gathering bacteria while excluding all contaminations from said plant and medium.

3. The process of developing a bacteriological culture, which consists in growing a plant inoculated with the desired bacteria within a germ proof inclosure.

4. The process of developing a bacteriological culture, which consists in growing a plant inoculated with the desired bacteria, within a germ proof inclosure, while maintaining a restricted supply of sterilized air to said inclosure.

5. The process of developing a bacteriological culture, which consists in growing a plant inoculated with the desired bacteria within a transparent germ proof inclosure.

6. The process of developing a bacteriological culture, which consists in growing, in a substantially transparent medium, a plant inoculated with the desired bacteria while excluding all contaminations from the plant and medium by a transparent germ proof inclosure.

7. The process of developing a bacteriological culture, which consists in planting, in a sterilized medium, within a germ proof inclosure, a vital seed free from contaminating germs maintaining a supply of sterilized air to said inclosure and supplying sterilized water to the plant.

8. The process of developing a bacteriological culture, which consists in growing a plant inoculated with the desired bacteria, within a germ proof inclosure, and then inoculating a medium with a portion of the roots of said plant.

9. The process of developing a bacteriological culture, which consists in growing a plant inoculated with the desired bacteria, in a medium within a germ proof inclosure and then inoculating another medium with a portion of the roots of said plant.

10. The process of developing a bacteriological culture, which consists in growing a plant, inoculated with the desired bacteria, within a germ proof inclosure, removing a nodule from said plant and inoculating a medium with the same.

11. The process which consists in preparing a composite culture consisting of a plurality of kinds of nitrogen gathering bacteria, then inoculating seed with such composite culture and planting the seed.

12. The process which consists in inoculating seed with a composite culture consisting of a plurality of kinds of nitrogen gathering bacteria, one of which kinds is specific to said seed, and then planting the seed.

13. The process which consists in inoculating the medium with a plurality of different kinds of nitrogen gathering bacteria to form a composite culture, and then preparing from said composite culture the cultures to be distributed.

14. The process of producing a bacteriological culture, which consists in growing a culture on a substantially solid medium and then adding a liquid to the solid medium.

15. The process of producing a bacteriological culture, which consists in first preparing a substantially solid medium, in a distributing vessel, inoculating it with nitrogen gathering bacteria, maintaining the medium under the required condition to develop the culture, then adding water to the top of the medium and finally sealing said distributing vessel.

16. A bacteriological culture comprising a medium, a plant growing in said medium and inoculated with the desired bacteria, in combination with a germ proof receptacle inclosing the plant and medium.

17. The combination, with a transparent germ proof receptacle, of a medium within said receptacle, and a plant inclosed by said receptacle and rooted in the medium, said plant being inoculated with the desired bacteria.

18. The combination, with a germ proof receptacle provided with means for maintaining a supply of sterilized air to its interior, of a medium within said receptacle, and a plant inoculated with the desired bacteria inclosed by said receptacle and rooted in the medium.

19. The combination, with a germ-proof receptacle pervious to air, of a medium within said receptacle, and a plant inoculated with the desired bacteria inclosed by said receptacle and rooted in the medium.

20. The combination, with a germ-proof transparent receptacle, of a substantially transparent medium within said receptacle and a plant inoculated with the desired bacteria inclosed and protected against contaminations by said receptacle and rooted in the medium.

21. The combination, with a glass vessel having an air-inlet and an air filter in said inlet, of a medium in said vessel, and a plant inoculated with the desired bacteria inclosed by the vessel and rooted in the medium.

22. The combination, with a vessel, having an air inlet and a lateral opening, a closure for said opening and an air filter in the air inlet, a medium in said vessel and accessible through the lateral opening and a plant inoculated with the desired bacteria, inclosed by the vessel and rooted in the medium.

23. A bacteriological culture comprising a plant inoculated with the desired bacteria, in combination with a germ-proof receptacle surrounding said plant and provided with means for permitting access to the roots of said plant.

24. A bacteriological culture comprising a medium, a plant growing in said medium and inoculated with the desired bacteria, in combination with a germ-proof receptacle inclosing the plant and medium and provided with means for permitting access to the roots of said plant.

25. A distributing package of nitrogen gathering bacteria containing a plurality of kinds of such bacteria.

26. A distributing package of nitrogen gathering bacteria comprising a composite culture of several kinds of virulent nitrogen gathering bacteria.

27. A distributing package comprising a sealed receptacle containing a substantially solid and also a liquid medium and nitrogen gathering bacteria in said media.

28. A distributing package comprising a receptacle, means for restricting the access of air to said receptacle, a substantially solid medium in said receptacle, a liquid upon said solid medium and a colony of nitrogen gathering bacteria in said medium.

29. A distributing package comprising a receptacle, means for restricting the access of air to said receptacle, a substantially solid medium in said receptacle, a liquid upon said medium, and a composite colony of different kinds of nitrogen gathering bacteria in said medium.

30. The combination, with a germ-proof receptacle provided with means for maintaining a supply of sterilized air to its interior, of a nitrogen-free medium within said receptacle and a plant inoculated with nitrogen-gathering bacteria inclosed by said receptacle and rooted in the medium.

31. A bacteriological culture comprising a nitrogen-free medium, a plant growing in said medium and inoculated with nitrogen gathering bacteria, in combination with a germ-proof receptacle inclosing the plant and medium, said receptacle being provided with means for maintaining a supply of sterilized air to its interior and with means for permitting access to the roots of said plant.

32. The process of developing a bacteriological culture, which consists in growing in a non-nitrogenous medium, a plant inoculated with nitrogen-gathering bacteria within a germ-proof inclosure, while maintaining a restricted supply of sterilized air to the interior of said inclosure, removing a nodule from said plant and inoculating a medium with said nodule.

33. The process of producing a bacteriological culture, which consists in first preparing a substantially solid medium, in a distributing vessel, inoculating it with nitrogen gathering bacteria, maintaining the medium under the required condition to develop the culture, then adding liquid to the top of the medium and finally sealing said distributing vessel.

34. A distributing package comprising a receptacle, a medium within said receptacle and a plurality of kinds of bacteria in said medium, one of said kinds of bacteria being a nitrogen-gathering bacteria specific to the particular plant on which the culture is to be used.

In testimony whereof I hereunto affix my signature in the presence of two witnesses.

GEORGE HERBERT EARP-THOMAS.

Witnesses:
M. C. MASSIE,
E. O. HILDEBRAND.

UNITED STATES PATENT OFFICE.

GEORGE H. EARP-THOMAS, OF BLOOMFIELD, NEW JERSEY.

MEANS FOR DISTRIBUTING SOIL BACTERIA.

1,099,121. Specification of Letters Patent. Patented June 2, 1914.

No Drawing. Application filed June 29, 1908. Serial No. 441,018.

To all whom it may concern:

Be it known that I, GEORGE H. EARP-THOMAS, a British subject, residing at Bloomfield, county of Essex, and State of New Jersey, have invented certain new and useful Improvements in Means for Distributing Soil Bacteria, fully described in the following specification.

This invention relates to the distribution of beneficial soil bacteria.

The desirability and practical necessity of having soils for growing all kinds of plants and crops supplied with the various species and varieties of beneficial soil bacteria is very generally recognized. Efforts to supply such bacteria to soils which are naturally deficient in them have been directed mainly to supplying the particular species of nitrogen fixing, or nitrifying, bacteria which enter the roots of leguminous plants usually forming nodules or swellings on the roots containing colonies of the bacteria, and which, like other species of nitrifying bacteria which exist independently of growing plants, fix atmospheric nitrogen in a form assimilable by plants. Two methods of supplying these nodule-forming nitrifying bacteria to soils which are naturally deficient in them have been heretofore followed; one being by distributing on the land soil from fields containing the desired bacteria, and the other by inoculating the soil or the seeds before planting with pure laboratory prepared cultures of the desired bacteria. The first of these methods, that is, the method of soil transfer, is objectionable because of the danger of spreading weeds, destructive crop diseases and harmful bacteria.

The object of the present invention is to provide an improved method and means for distributing nodule-forming nitrifying bacteria and other kinds of beneficial soil bacteria whereby any advantages belonging to the soil transfer method of inoculation, above referred to, are retained, and the disadvantages and objections to such method of inoculation entirely avoided and overcome. To this end, I propose to distribute for inoculation purposes, soil which has been freed from weed seeds and all harmful germs and bacteria, and which is inoculated with artificially or laboratory prepared pure cultures of the desired beneficial bacteria.

My method of preparing such inoculated soil is briefly as follows:—I take a quantity of soil which is favorable to the growth of the bacteria and treat the same to kill all weed seeds, disease germs and all organisms which it may contain. For this purpose, the soil is preferably sterilized by steam or dry heat, preferably the latter. A practical method of sterilizing the soil is to pass it in an even layer slowly over a sufficiently heated iron sheet, the soil being stirred and turned as it is passed over the heated surface. It may also be sterilized by the use of any suitable form of apparatus, such as is used for roasting ores or minerals; as, for example, a revolving cylinder heated to the desired degree provided with a spiral guide or flange on its interior surface by which the soil will be advanced gradually through the cylinder and at the same time agitated and stirred so as to insure the proper heating of all parts of the same. The soil thus sterilized and freed of its weed seeds, all weed seeds which it contains having been killed by the heat, is then inoculated with the desired beneficial bacteria. Artificially prepared pure cultures are used for this purpose, and the inoculation is preferably performed by mixing with the sterilized soil a more or less diluted solution of the culture, the amount of bacteria used depending upon the amount of the inoculated soil which is to be used for inoculating a given area of land.

If soil of the proper character and composition is used, the bacteria will thrive in the same, increasing in numbers and remaining in an active and virile condition for a considerable length of time, and the inoculated soil may be shipped to all parts of the world with the certainty that it will reach its destination in good condition.

The inoculated soil may be sown on the land like seed in the condition it is received by the user, but a preferred method of using it is to mix the amount of soil furnished for an acre of ground with, say, a cartload of soil from the field to be inoculated and then spread such soil evenly over the field, harrowing it in as soon as possible.

The soil to be sterilized and inoculated should be of a character favorable to the growth of the desired bacteria, containing the necessary elements for sustaining the bacterial life, but almost any ordinary garden soil may be used. It is preferable, however, to use a non-nitrogenous soil, or one which is free, or substantially free from nitrogen. This for the same purpose that it is desirable in growing laboratory cultures

to use a non-nitrogenous medium, that is, to cause and accustom the bacteria to take the nitrogen which they require from the air rather than to feed on nitrogen contained in the soil. If the soil is lacking in carbonates or carbo-hydrates or other elements or substances which the bacteria require, they should be supplied. As the soil is to be quite heavily inoculated with bacteria it should contain a comparatively large supply of carbonaceous matter, and if the carbonaceous matter is not in the soil in sufficient quantities this is corrected preferably by the addition of nitrogen-poor humus or clay-loam, or better, both clay-loam and humus; or the elements lacking may be supplied in chemical form. Ordinary garden soil with the addition of say 10 to 20 per cent. of humus and clay-loam will usually be found to contain all necessary substances for the growth of the bacteria. Of course much larger relative quantities of these substances may be used if desired, or clay-loam alone or a mixture of clay-loam and nitrogen-poor humus would answer the purpose well.

When humus and clay-loam, or either of them, is added to the soil, they may be added before sterilization, and the combined soil then treated to sterilize it and kill the weed seeds; or the constituents may be separately treated. A good method is to sterilize clay-loam which is to be added to the soil separately from the main body of the soil, and then to inoculate it and mix the moistened and inoculated clay-loam with the main portion of the sterilized soil. When the sterilizing is done by dry heat, it is sometimes desirable to add carbo-hydrates in the form of sugar, maltose or its equivalent to the soil after it is sterilized. Unless carbonaceous matter is to be added after sterilization, the temperature during sterilization must not be high enough to destroy the carbonaceous matter in the original soil.

For most forms of the nodule-forming bacteria, the soil should be neutral or slightly alkaline, and if the soil to be used should be acid, this should be corrected by the addition of a suitable quantity of lime, preferably in the form of carbonate of lime. With regard to the physical condition of the soil, it is desirable that it be of a light character to admit air readily, friable, of fine texture, and of an absorbent nature.

The soil may be inoculated with a single species or variety of beneficial bacteria, or with a plurality of species or varieties. Soil for inoculating land for growing a particular species of legume may be inoculated with only the particular kind or variety of nodule-forming bacteria corresponding to the particular legume; or the soil may be inoculated with the various kinds or varieties of nodule-forming bacteria belonging to the commonly used legumes, either by inoculating with separate cultures of each different variety of the bacteria or with a composite culture containing the various varieties of these nodule-forming bacteria with which the sterilized soil is to be inoculated. Such composite cultures for inoculating the soil may be produced as set forth in United States Letters Patent No. 865,965, granted to me September 10th, 1907. Soil inoculated with nodule-forming bacteria specific to the several legumes may be used for inoculating land for growing any of such legumes or their closely related species. In order to produce an inoculated soil for inoculating land for growing various kinds of legumes, it will not be necessary in practice to inoculate with the specific bacteria for each of the various species of legumes to be grown, but it will usually be sufficient to inoculate with bacteria specific to one or more species of each of the different groups of closely related species of legumes. For example, soil inoculated with bacteria specific to red clover would answer for inoculating land for growing alsike or white clover.

In preparing inoculated sterilized soil for inoculating land for growing legumes, I prefer to inoculate the soil not only with the nodule-forming bacteria, but also with other kinds of beneficial soil bacteria, such as the independent or non-symbiotic nitrifying bacteria, and the peptonizing and ammonifying bacteria.

The use of inoculated soil prepared in accordance with this invention is, of course, not limited to the inoculation of land for growing leguminous crops. Sterilized soil may be inoculated with other forms of bacteria such as have been already isolated or which may hereafter be isolated which are specific to various crops other than legumes; and soil inoculated with the independent forms of nitrifying bacteria and preferably also with the peptonizing and ammonifying bacteria or other forms of beneficial bacteria will be found to be of value for general use for inoculating land which is deficient in such bacteria. It is also desirable to have the inoculated sterilized soil prepared for such general use inoculated with the nodule-forming or legume bacteria.

While for most forms of beneficial bacteria the soil should be, as before stated, neutral or slightly alkaline, yet certain forms of nodule-forming bacteria specific to certain legumes thrive best in a slightly acid medium or soil, and in order to get the best growth and most marked results from these particular bacteria the soil to be inoculated should preferably be in a slightly acid condition. The bacteria specific to cow peas, for example, thrive best in a soil which is slightly acid. Soil to be inoculated with the bacteria specific to cow peas, therefore, should not be limed, but should be allowed

to remain slightly acid, or should be made so by the addition of a suitable substance such as acid sulfates or acid phosphates. Most of the legumes, however, and their corresponding bacteria thrive best on a neutral or slightly alkaline soil, as above stated. Some, moreover, do slightly better on a soil just neutral, while others are favored by a slightly alkaline condition of the soil. I prefer, therefore, in preparing inoculated soil to be used for inoculating land for growing the various species of legumes to use soil of three different qualities with respect to their acid or alkaline condition, that is, I would use a slightly alkaline soil for inoculating for legumes which thrive best in an alkaline soil; a neutral soil for legumes which thrive best in a neutral soil; and a slightly acid soil for legumes which thrive best in a slightly acid soil.

A good method of securing the best soil to use in preparing inoculated sterilized soil in accordance with this invention, is to select soil for a particular species or group of legumes from a locality where legumes of that particular species or group have grown well and have been well supplied with healthy root nodules. After being sterilized and inoculated, the soil should be protected against contamination, especially against access of molds and other harmful organisms. The degree of protection required while being stored and during shipment will depend largely on the amount of moisture in the inoculated soil. If the soil is quite dry, molds and other organisms will not readily penetrate into it and it could be shipped in bags or ordinary boxes. If the soil is too dry, however, the growth of the beneficial bacteria will be retarded, and it is desirable to have the soil moistened sufficiently so that its condition will be favorable for their growth and development. In such moistened condition, however, molds and other harmful organisms are more liable to develop on and spread through the soil, and such moistened inoculated soil should, therefore, be sealed against access of molds and other harmful organisms in suitable receptacles for shipment, and preferably should be sealed immediately after inoculation. It is desirable that inoculation should take place as soon as the soil is cooled sufficiently after being sterilized and without its having been allowed to become contaminated. If the inoculated soil is to be kept for a considerable length of time after being sealed against access of molds and other harmful organisms, or of air laden with such, provision is preferably made for allowing sterilized air to enter the receptacle containing the inoculated soil. This may readily be accomplished by providing the receptacle with an opening which is filled with properly compacted sterilized cotton.

A good way of distributing the inoculated sterilized soil is to have a small quantity of such soil which is in a moistened condition surrounded by dry sterilized soil, which dry soil may be uninoculated sterilized soil, but which preferably will also be inoculated. The soil may be packed in this way by putting a layer of the dry soil in the bottom of a box or other receptacle, placing the moist soil on the center of said layer and then filling up the box with the dry soil. The moist soil in the center of the box will thus be entirely surrounded by dry soil. Another way of securing this result is to pack dry sterilized soil in a receptacle in which it is to be shipped and then by means of a suitable spray, syringe or other suitable instrument to inject into the center of the dry soil a sufficient quantity of liquid culture to moisten and inoculate the soil at and near the center of the package. The advantage of this method of distributing inoculated soil, that is, with moist soil surrounded with dry soil, is that the moist soil in the center will be in a condition most favorable for the development and growth of the bacteria and will be protected by the dry soil against molds and other harmful organisms. When packed in this way, the inoculated soil may be safely distributed over long distances and kept for a long time when packed in ordinary wooden boxes or even in bags.

What is claimed is:—

1. As a new article of manufacture, substantially nitrogen-free soil freed of weed seeds and harmful organisms but containing available carbonaceous matter, and artificially inoculated with beneficial soil bacteria.

2. As a new article of manufacture, substantially nitrogen-free soil containing clay and available carbonaceous matter and freed from weed seeds and harmful organisms, and artificially inoculated with beneficial soil bacteria.

3. As a new article of manufacture, soil composed of a mixture of garden soil, humus and clay-loam, the quantity of the humus and clay-loam being less than the quantity of garden soil in the mixture, and said mixed soil being freed from weed seeds and harmful organisms, and inoculated with beneficial soil bacteria.

4. As a new article of manufacture, substantially nitrogen-free soil which has been sterilized by heat, containing available carbonaceous matter and inoculated with beneficial soil bacteria.

5. As a new article of manufacture, sterilized substantially nitrogen-free soil containing available carbonaceous matter and inoculated with pure cultures of beneficial soil bacteria.

6. As a new article of manufacture, sterilized substantially nitrogen-free soil containing available carbonaceous matter and inocu-

lated with pure cultures of nitrifying bacteria.

7. As a new article of manufacture, sterilized substantially nitrogen-free soil containing available carbonaceous matter and inoculated with pure cultures of nodule-forming nitrifying bacteria.

8. As a new article of manufacture, sterilized substantially nitrogen-free soil inoculated with pure cultures of a plurality of kinds of nitrifying bacteria.

9. As a new article of manufacture, sterilized substantially nitrogen-free soil inoculated with pure cultures of a plurality of kinds of nodule-forming nitrifying bacteria.

10. As a new article of manufacture, sterilized soil inoculated with pure cultures of nodule-forming nitrifying bacteria and independent or non-symbiotic nitrifying bacteria.

11. As a new article of manufacture, sterilized soil inoculated with nitrifying, peptonizing and ammonifying bacteria.

12. As a new article of manufacture, sterilized soil inoculated with nodule-forming and independent or non-symbiotic nitrifying bacteria, peptonizing bacteria and ammonifying bacteria.

13. A new article of manufacture comprising substantially nitrogen-free soil freed of weed seeds and harmful organisms and inoculated with beneficial soil bacteria, and a protective envelop comprising pulverulent material inclosing said soil and preventing access of molds and other harmful organisms thereto.

14. A new article of manufacture comprising sterilized substantially nitrogen-free soil inoculated with beneficial soil bacteria, and a protective envelop of inorganic material pervious to air inclosing said soil and preventing access of molds and other harmful organisms thereto.

15. As a new article of manufacture, a body of soil comprising soil inoculated with pure cultures of beneficial soil bacteria, a localized portion of said body having a greater moisture content than and being protected by the remainder of said body.

16. A new article of manufacture comprising moist sterilized soil inoculated with beneficial soil bacteria, a layer of dry soil enveloping said moist soil and a closed receptacle inclosing both said soils and having an air-supply opening filled with compacted sterilized cotton.

17. As a new article of manufacture, a body of moist sterilized soil inoculated with beneficial soil bacteria and surrounded by dry sterilized soil.

18. As a new article of manufacture, a body of moist sterilized soil inoculated with beneficial soil bacteria and protected by dry sterilized soil.

19. As a new article of manufacture, a body of moist sterilized soil surrounded with dry sterilized soil, both the moist and the dry soil being inoculated with beneficial soil bacteria.

20. As a new article of manufacture, a body comprising both moist and dry soil in substantially separate arrangement, said soil being freed of weed seeds and harmful organisms and inoculated with beneficial soil bacteria, said moist soil being protected by said dry soil.

21. As a new article of manufacture, a moist medium inoculated with bacteria and surrounded by a dry powdered material.

22. As a new article of manufacture, a moist medium inoculated with bacteria and protected by a dry powdered material.

In testimony whereof, I have hereunto set my hand, in the presence of two subscribing witnesses.

GEORGE H. EARP-THOMAS.

Witnesses:
HARRY S. CONNOLLY,
A. L. KENT.

Copies of this patent may be obtained for five cents each, by addressing the "Commissioner of Patents, Washington, D. C."

UNITED STATES PATENT OFFICE.

WILLIAM BEECROFT BOTTOMLEY, OF LONDON, ENGLAND.

TREATMENT OF PEAT FOR MANURIAL AND OTHER PURPOSES.

1,106,275. Specification of Letters Patent. Patented Aug. 4, 1914.

No Drawing. Application filed July 15, 1913. Serial No. 779,168.

To all whom it may concern:

Be it known that I, WILLIAM BEECROFT BOTTOMLEY, a subject of the King of Great Britain, residing in London, England, and whose post-office address is King's College, in the county of London, England, have invented a certain new and useful Improved Treatment of Peat for Manurial and other Purposes, of which the following is a specification.

According to this invention peat can be converted into an excellent manure by treating it with micro-organisms capable of producing ammonia. Such micro-organisms are obtainable by known methods from ordinary soil or from other sources such as putrefying bouillon. There are several species of them, such as *Bacillus mycoides*, *Bacillus subtilis*, *Bacterium aerogens* and *Bacterium fluorescens liquefaciens*. It is not essential to use a pure culture of any particular species or of the mixed species, since other micro-organisms may be present. The effect of these micro-organisms on the peat is to convert the humic acid and humous bodies contained in it into compounds soluble in water and at the same time to disintegrate the peat so that it is readily distributed.

A product richer in nitrogen is obtained if nitrogen fixing organisms such as *Azotobacter* and *Bacillus radicicola* are present in the mass.

In addition to its usefulness as a manure the product forms a ready source of the substances commonly classed as humic acid or humous bodies, which have lately found application in industry.

There is no difficulty in obtaining a culture of micro-organisms, containing no doubt very many species, suitable for my invention. For this purpose 10–20 grams of fertile soil may be added to a culture medium comprising 1 liter of water, 20 grams of dextrin, 1 gram of dipotassium phosphate, ½ gram of magnesium sulfate, 2 grams of calcium carbonate and 10 cc. of bouillon. The scum which forms on the surface of the liquid in the course of a few days is suitable for my invention. Pure culture consisting of any of the individual species that are active for the purpose or mixed cultures may of course be prepared, but this is not necessary so far as is at present known to me.

To the peat or peat material is added water containing the organisms and the mass is allowed to undergo the change involved, for some days. When the peat has been dried, as is the case with certain prepared peat, the amount of water added should suffice to moisten the mass thoroughly. It is not, however, necessary to dry the peat, for if this is in its natural wet condition it may be merely sprinkled with the water containing the mirco-organisms.

The process is preferably hastened by adding to the mass a nitrogenous organic material, particularly a weak solution of a soluble nitrogenous organic material, such as albumin, gelatin or meat extract; a solution containing say 0.25–0.5 per cent., of any standard meat extract, for example will serve. Such a solution may constitute the liquid in which the micro-organisms may be contained as aforesaid. I have found that a very suitable nitrogenous extract consists of the waste liquor obtained from the boiling of bones. It is also desirable to add a small proportion of a carbohydrate such as sugar or starch; say about 0.1 per cent. of the dry weight of the original peat, dissolved or suspended in a little water.

The saturated peat may be left at a temperature of 24–30° C. for three weeks or thereabout and may then be dried. In this condition it may be applied directly as a manure. Or before it is dried it may be sterilized, such as by live steam, and then further treated with nitrogen-fixing organisms for increasing the amount of nitrogen in the mass, these organisms being capable of growing in the altered peat.

If soluble humous substances be required the treated mass may be leached with water and the solution used directly for any of the purposes to which humus is applied; or an acid may be added to precipitate the humic acid from the aqueous solution. The aqueous extract of the treated peat is also useful as a liquid manure.

Having thus described the nature of my said invention and the best means I know of carrying the same into practical effect, I claim:—

1. A process of treating peat which consists in adding to the moist peat aërobic micro-organisms capable of producing ammonia and allowing these micro-organisms to multiply in the peat.

2. A process of treating peat which consists in adding to the moist peat aërobic

micro-organisms capable of producing ammonia and nitrogen-fixing micro-organisms and allowing the said micro-organisms to multiply in the peat.

3. A process of treating peat which consists in adding to the peat water containing ammonia-fixing micro-organisms and drying the material after some days.

4. A process of treating peat which consists in adding to the peat water containing ammonia-fixing micro-organisms and nitrogen-fixing micro-organisms and drying the material after some days.

5. A process of treating peat which consists in adding to the peat water containing ammonia-fixing micro-organisms, then sterilizing the material and adding to it nitrogen-fixing micro-organisms.

6. A process of treating peat which consists in adding to the peat nitrogenous material adapted to be converted in part into ammonia, then adding to the moist peat ammonia-producing micro-organisms and drying the material after some days.

7. As a new product peat which has been subjected to the action of ammonia-producing micro-organisms and contains soluble humates.

In testimony whereof I have signed my name to this specification in the presence of two subscribing witnesses.

WILLIAM BEECROFT BOTTOMLEY.

Witnesses:
JOSEPH MILLARD,
GEO. J. M. FRANKLIN.

UNITED STATES PATENT OFFICE.

GEORGE H. EARP-THOMAS, OF GLEN RIDGE, NEW JERSEY.

BACTERIAL PRODUCT AND PROCESS OF PREPARING SAME.

,252,332. Specification of Letters Patent. Patented Jan. 1, 1918.

Drawing. Application filed March 30, 1917. Serial No. 158,625.

o all whom it may concern:

Be it known that I, GEORGE H. EARP-HOMAS, formerly a citizen of New Zealand, t who have declared my intention of be-ming a citizen of the United States, resid-g at Glen Ridge, in the county of Essex d State of New Jersey, have invented cer-in new and useful Improvements in Bac-rial Products and Processes of Preparing me; and I do hereby declare the following be a full, clear, and exact description of e invention, such as will enable others illed in the art to which it appertains to ke and use the same.

This invention relates to bacterial prod-ts and processes of preparing same; and particular it relates to bacterial products mprising inoculated material, most desir-ly in the form of grains or granules, ated or covered with a protective substance such manner as to afford protection to id inoculated material from injury or con-mination to which it may be exposed by ason of its environment. The invention especially useful when it is desirable to sociate a bacterial culture with material such character or chemical reaction as uld exercise an injurious or unfavorable ect on the unprotected culture.

The difficulty of preventing infection or ntamination of bacterial cultures by for-n micro-organisms, especially when the tures are to be used or kept outside of a oratory or transported from place to ce, is well known. Many types of bac-ia such as the nitrogen-gathering or ni-gen-fixing bacteria (*Pseudomonas radi-ola*) infesting the roots of legumes, for mple, are aerobic, and hence any means pted for exclusion of foreign and harm-micro-organisms from culture of such teria must nevertheless permit access of to the cultures. For the convenient com-rcial storage and transport of such cul-es *en masse*, a container of the character closed in my Patent 1,137,388 of April 1915, has proved eminently satisfactory practice; but, as will hereinafter appear, s sometimes desirable to transport or to ploy such a culture in the form of rela-ly small discrete bodies, pieces, or gran-, and in such cases the present invention be practised to great advantage.

n accordance with the present invention, ody consisting of or comprising a bac-al culture is protected from infection and contamination by providing said body with an adherent protective coating of a suitable substance which in itself is substantially indifferent toward the culture, or which at any rate does not deleteriously affect the same, said substance being of such nature as to shield the culture from contact with substances to which it would otherwise be exposed by reason of its environment. More particularly, the invention is directed to protecting in this way small bodies, particles, or granules, of suitable material which has been inoculated with beneficial micro-organisms, such as beneficial soil bacteria. As typical of such beneficial soil bacteria may be mentioned ammonifying bacteria, which convert the insoluble nitrogen of organic matter into ammonia; nitrifying bacteria, (*e. g. Nitrosomonas*) which convert ammonia into nitrous acid; nitrate bacteria (nitrobacter), which carry the oxidation to the nitrate stage; and nitrogen-fixing bacteria, both symbiotic (*e. g. Pseudomonas radicicola*) and non-symbiotic (azotobacter); as well as other bacteria which I have found to grow vigorously in nitrogen-free media and which are useful in soil improvement, but which have not all been individually isolated and identified. An especially desirable result attainable by means of my invention is that granular inoculated material, provided with a protective coating of the general character described, can be mixed with other fertilizer materials, especially with chemical fertilizers having an acid or basic reaction of such character or intensity as would interfere with or inhibit the growth and development of unprotected beneficial micro-organisms. Cultures coated in accordance with the invention retain their activity and virulence unimpaired under conditions which would destroy or seriously affect cultures not thus protected.

While particular advantages are realized in applying the principles of the invention to the manufacture of mixed fertilizers in the manner mentioned, it is to be understood that the invention is capable of other applications, as will appear more fully hereinafter, and that it is therefore not limited to the fertilizer art. In order to explain the principles of the invention more fully by way of a concrete example, however, the application thereof to the preparation of a mixed fertilizer will be described in detail.

Cultures of bacteria suitable for use in

practising the present invention may be prepared in any suitable way, but the methods described in my prior Patents 816,850 and 865,965 are especially desirable. Pure cultures or mixed cultures of bacteria or other micro-organisms, having thus been prepared in any suitable manner, and most advantageously in a highly virulent condition, are used for the inoculation of specially prepared fine granular material which has been thoroughly mixed and dried out to a desirable point, which point is, however, considerably short of complete desiccation. This granular material is desirably a substance such as peat, humus, or muck, which may be generically termed humus material or humus-like material, although any other suitable material for the growth of bacteria may be employed, such as comminuted wood fiber for example. For several reasons, humus material is especially valuable for the purposes in view. It is nitrogen-free in the sense that it contains substantially no nitrogen in a form directly available to nitrobacteria; and this is important in maintaining the bacteria virulent. On the other hand, it contains nitrogen in insoluble form which is convertible into ammonia, and therefore eventually made available, by ammonifying bacteria, especially in the presence of suitable basic or antacid substances. Furthermore, humus material is naturally very high in hydroscopic moisture content which it retains tenaciously, samples of peat which have been thoroughly air-dried very commonly containing from 20 to 40 per cent. of hydroscopic moisture. Since a moist medium is essential to continued virulence of bacteria, this characteristic of humus material is of very great importance for present purposes.

If not in itself adapted to serve as food for bacteria, the granular carrier material can be made suitable for the growth of the bacteria by impregnating or otherwise supplying it with soluble nutrients, such as malt sugar, sucrose, etc., and adding certain inorganic agents such as potassium phosphate, magnesium sulfate, wood ashes, all compounded to suit the particular type of micro-organisms to be grown. The granulated carrier is inoculated before or after addition of nutrients with the prepared pure or mixed cultures by methods well known in the art, and the material is then treated with a suitable protective material in such manner as to provide the bacteria with the requisite protection. As protective agents, a large number of materials are satisfactory and readily available. Speaking generally, it is desirable that the coating agent employed be such as will give a very thin tough and closely adherent coating. Agar-agar, and the seaweed jellies generally, give this type of coating and are therefore especially well adapted for the purpose. Among other coating and binding agents useful under certain circumstances in carrying out the invention may be mentioned colloidal material generally, so-called soluble celluloses, gums, resins, waxes, carbohydrates incuding starches, dextrin, glucose, etc., gelatin, glue, clays and the like. These coating agents may be used individually or in various combinations. Thus, for example, a compound coating of gums or glues and a syrup has certain advantages; and a mixture of waxes and syrups works well.

These various coating agents give coatings whose permeability to air, while differing considerably is sufficient for the purposes of the invention. Aerobic bacteria, when grown in a confined space, soon reduce the pressure therein below atmospheric, and eventually a rather high degree of vacuum is attained. For this reason the inclosed bacteria can draw their required air supply through a coating which is only very slightly pervious to air.

The actual operations involved in preparing coated cultures according to the invention may vary considerably, but a typical and desirable form of procedure as carried out in practice for producing coated nitrobacteria cultures is substantially as follows: A good quality of peat or other humus material is dried and sifted to secure a fine granular carrier material of uniform character. As before pointed out, peat or humus material broadly is especially suited for the purposes of the invention because it contains nitrogen which though ordinarily unavailable, can readily be made available as food for beneficial soil bacteria, especially nitro-bacteria. For this reason and also because, generally speaking, a mildly alkaline reaction favors the growth of the bacteria, the reaction of the peat, if acid, may be corrected by adding thereto the proper amount of lime or other alkaline or antacid agent. The addition of wood ashes is particularly to be recommended. The peat may be rendered still more favorable to bacterial growth by addition of other bacterial food and stimulants as above set forth.

After this preliminary treatment, or simultaneously therewith, the humus material is inoculated; and in this typical example it is inoculated with a mixed culture of nitro-bacteria, comprising ammonifying bacteria, nitrifying bacteria, and nitrogen-fixing bacteria (*e. g. Pseudomonas radicicola* and azotobacter), this combination of bacteria having been found especially advantageous in practice. Other beneficial nitrogen-fixing bacteria above referred to as not specifically identified, but which are obtainable from various kinds of soil and which I have found to exercise a distinctly favorable effect on the development of other nitro-bacteria

when associated therewith, may be included in the mixed culture to advantage, but this is not essential. One way of conveniently accomplishing the inoculation is by impregnating the prepared peat with a liquid nutrient medium containing the mixed culture grown, for example, by the methods of my prior patents aforesaid. The inoculated peat is next dried cautiously in a suitable pan until the particles or granules of peat will freely separate; but complete drying or desiccation of the material is to be avoided, for reasons before explained. In using peat, however, there is not much danger of excessive drying because of the tenacity with which peat holds moisture. The pan is then revolved, and the operation of coating the granules begins. In the present example, agar-agar is employed as the coating agent. Liquid agar-agar jelly is prepared in the usual manner by heating agar-agar with water until a readily fluent homogeneous mixture is obtained. After cooling to a temperature at which the bacteria will not be injured, say about blood heat (37° C.), the liquid jelly is gradually poured into the revolving pan until the humus granules have taken up about all they will. The mass is then partly dried in the revolving pan to remove excess moisture, yielding granules both impregnated and coated with agar-agar. Most desirably the granules are given two more coatings in the same way already described for the first coat. A little drying powder, such as powdered talcum or chalk, added to the mass in the pan, and application of a warm air blast serve to hasten the drying of the mass. Talcum and chalk are also useful to prevent the coated granules from sticking together and to cover the final coating thoroughly. It is desirable also to add a little powdered boric acid or borax to the material during the drying. This does not hurt the inclosed bacteria and is effective in keeping the material from molding while drying out and before it is placed in cans for shipment. Furthermore boric acid and borax also act as good lubricants in the pan, separating the granules as they are being dried and preventing their sticking to the pan and to each other.

The coating obtained by the use of agar-agar jelly in the manner described is particularly desirable in character. In drying, the agar-agar membrane shrinks down upon the particle or granule of material which it covers and clings to it tenaciously, conforming accurately to the contour of the protected surface. Moreover the coating is strong and tough, resistant, and neutral. When, as in the case of a fertilizer, the coated particle is applied to soil and exposed to a moist environment, the agar-agar coating does not immediately disintegrate, but first softens and swells while still affording protection to the inclosed culture which, however, is now free to propagate through the soft jelly wall and begin its beneficial action on the soil.

Where desirable a sugar solution or glucose, either of which serves admirably as a coating agent, may replace, or may be used in conjunction with, agar-agar in the procedure above described. They have several characteristics which render them valuable for certain purposes as coating agents, among which may be noted their solubility and their nutrient properties. By reason of the latter they serve not only as protective coating agents but also as bacterial foods; while their solubility insures that the contained bacteria cultures shall be very promptly freed for action when the coated material is placed in a moist environment.

The granular inoculated material, when coated as above described, may be packaged and distributed in this form. But it is especially useful for mixing with other fertilizing materials in the proper proportions to give a suitable mixed or complete fertilizer. Such additional fertilizing materials include, for example, lime, gypsum, dolomite, acid phosphate, muriate and sulfate of potash, magnesia, basic slag, phosphate rock, tankage, humus, manure, etc. In the mixed fertilizer prepared according to the present invention, the bateria are protected from direct contact with acid or alkali matter and their growth and development are therefore unimpeded. The dust or other portions of the acid or alkali chemicals or other injurious matter contained in the added fertilizer material will not penetrate and come into contact with the delicate structure of the micro-organisms inclosed in the coated granules or particles.

It is to be noted also that, in addition to the fact that the micro-organisms in the coated granules are shielded from undesirable contact with the admixed fertilizer materials, said admixed materials, if they happen to be distinctly acid or basic in reaction, act as a further protection to prevent access of undesirable foreign micro-organisms to the beneficial micro-organisms. The inoculated material is therefore protected by a material destructive to micro-organisms; but is nevertheless shielded from direct contact therewith.

As before stated, the invention is not limited to the mixing and distribution of beneficial bacteria or micro-organisms with chemical fertilizers. The method of coating bodies of inoculated material in the manner described constitutes broadly a desirable way of distributing bacteria and protecting them against contamination during transit, whether said inoculated material be accompanied or not by additional material of any kind. Coated bodies, pieces, gran-

ules, or particles of material inoculated with beneficial soil bacteria are useful alone for direct application to the soil. The coating not only acts as a protection during distribution, but it also acts as a protection in the soil against harmful micro-organisms. The beneficial bacteria are thus supplied with a nidus for growth from which they can propagate and produce their chemical products which are valuable in increasing the available nitrogen content of the soil and in producing acids which make available the potential plant foods of the soil itself.

The principles of this invention can also be extended to the distribution of beneficial micro-organisms for consumption by man and animals by preparing specially granulated material, inoculating it with suitable syrup solutions containing beneficial bacteria, and then coating the inoculated granules with a soluble protective material. The cultures are subsequently liberated during the process of digestion, the period required for liberation being dependent upon the solubility of the particular substance employed to coat the granular material.

While a practical way of carrying out the invention has been described above for the sake of a concrete example, it is to be understood that various changes and modifications may be introduced while still realizing the advantages of the invention to a greater or less extent. For instance, instead of proceeding as in the specific example given it is feasible to mix the inoculated carrier substance, such as peat or the like, with a protective binder such as agar-agar, syrups, etc., then to force the mixture through a perforated plate to granulate or comminute it, and finally to dry the comminuted material to a desirable extent, either with or without further treatment with protective material. The product contains active bacteria carried in a sufficiently moist medium and shielded by adherent protective material. This and all other forms of the method and product coming properly within the broad scope of the invention are contemplated herein.

What I claim is:

1. As a new article of manufacture, material inoculated with micro-organisms and protected by an adherent substance.

2. As a new article of manufacture, material inoculated with micro-organisms and provided with an adherent protective covering of colloidal material.

3. As a new article of manufacture, material inoculated with micro-organisms and coated with material of a gummy nature.

4. As a new article of manufacture, material inoculated with micro-organisms and coated with a soluble protective material.

5. As a new article of manufacture, inoculated material in granular form, protected by an adherent substance.

6. As a new article of manufacture, inoculated material in the form of discrete particles or granules, each provided with an adherent protective coating.

7. As a new article of manufacture, inoculated material in the form of discrete particles or granules, each provided with an adherent coating superficially treated to prevent the granules from sticking together.

8. As a new article of manufacture, material inoculated with beneficial micro-organisms and coated with agar-agar.

9. As a new article of manufacture, granular material inoculated with beneficial bacteria and protected by a substance of gum-like character.

10. As a new article of manufacture a culture of nitro-bacteria in active condition provided with an adherent coating of protective material.

11. As a new article of manufacture a relatively moist medium inoculated with nitro-bacteria in virulent condition and provided with an adherent coating of protective material.

12. A fertilizer composition comprising pieces or particles of inoculated material provided with an adherent protective coating.

13. A fertilizer composition comprising pieces or particles of material inoculated with bacteria beneficial to soil and coated with a protective substance, and additional fertilizer material mixed therewith.

14. A fertilizer composition comprising granular material inoculated with suitable nitro-bacteria and provided with a protective coating, and additional fertilizer material mixed therewith.

15. A fertilizer composition comprising granular material of relatively high moisture content inoculated with ammonifying, nitrifying, and nitrogen-fixing bacteria and provided with a protective coating, and chemical fertilizer material mixed therewith.

16. A fertilizer comprising the combination, with particles or granules of humus-like material, inoculated with ammonifying, nitrifying and nitrogen-fixing bacteria and coated with a protective substance, of additional fertilizer material mixed therewith.

17. As a new manufactured product, the combination, with suitable material inoculated with useful bacteria and protected by an adherent substance, of material antagonistic to bacterial growth surrounding such protected material.

18. As a new manufactured product, the combination, with particles of a suitable material, such as peat or humus, inoculated with beneficial soil bacteria and protected by an adherent substance relatively inert

aracter, of a fertilizer material admixed
h such protected particles and of a char-
er unfavorable to bacterial growth.
9. As a new manufactured product, the
nbination, with particles of a suitable ma-
ial, such as humus, inoculated with bene-
al soil bacteria and coated with a protec-
e substance relatively inert in character,
a mineral fertilizer of acid or basic re-
ion admixed with such coated particles.
0. The process of preparing useful bac-
ial products which comprises inoculat-
suitable material with micro-organisms,
d treating the inoculated material with an
esive protective substance.
1. The process of preparing useful bac-
ial products which comprises inoculating
table material with micro-organisms, and
ting the inoculated material with a
mmy or colloidal substance.
2. The process of preparing useful bac-
ial products which comprises inoculating
nular material, mixing a liquid coating
stance therewith, and drying the mixture
ile substantially maintaining its granular
m.
3. The process of preparing useful bac-
ial products which comprises inoculat-
granular material, mixing a liquid coat-
substance therewith, drying the mixture
adding a non-adhesive substance during
drying to maintain the granular char-
er of the product.
4. The process of preparing useful bac-
ial products which comprises prelimi-
ily treating humus material to render it
non-acid in reaction, adding a nutrient there-
to, inoculating the composition with a
mixed culture of several varieties of nitro-
bacteria, mixing a liquid protective material
with the inoculated mass, and drying under
proper conditions to secure a granular
product.

25. The process of preparing useful bac-
terial products which comprises prelimi-
narily treating humus material to render it
still more favorable to bacterial growth, in-
oculating it with a substantially liquid cul-
ture of ammonifying, nitrifying and nitro-
gen-fixing bacteria, drying until the inocu-
lated material granulates, and then coating
the granules with an adherent protective ma-
terial.

26. The process of preparing useful bac-
terial products which comprises prelimi-
narily treating humus material to render it
still more favorable to bacterial growth, in-
oculating it with a substantially liquid cul-
ture of ammonifying, nitrifying and nitro-
gen-fixing bacteria, drying until the inocu-
lated material granulates, and then coating
the granules with agar-agar.

27. The process of preparing a mixed fer-
tilizer which comprises inoculating a suitable
material with beneficial soil bacteria, coating
the inoculated material with a protective
substance, and mixing the resultant product
with other material having fertilizing value.

In testimony whereof I hereunto affix my
signature.

GEORGE H. EARP-THOMAS.

UNITED STATES PATENT OFFICE.

GEORGE H. EARP-THOMAS, OF GLEN RIDGE, NEW JERSEY

FERTILIZER AND PROCESS OF MAKING SAME.

1,309,723. Specification of Letters Patent. Patented July 15, 1919.

No Drawing. Application filed April 10, 1917. Serial No. 160,960.

To all whom it may concern:

Be it known that I, GEORGE H. EARP-THOMAS, formerly a citizen of New Zealand, but who have declared my intention of becoming a citizen of the United States, residing at Glen Ridge, in the county of Essex and State of New Jersey, have invented certain new and useful Improvements in Fertilizers and Processes of Making Same; and I do hereby declare the following to be a full, clear, and exact description of the invention, such as will enable others skilled in the art to which it appertains to make and use the same.

This invention relates to fertilizers and processes of making same; and it comprises growing suitable nitro-bacteria in the presence of material, especially a phosphatic or potassiferous mineral, containing plant food in unavailable form, whereby such material is modified and rendered suitable for fertilizer purposes.

In particular the invention is concerned with the treatment of raw phosphate rock, *e. g.*, apatite, phosphorite, "land rock", etc., with humus, peat, muck, or the like, carrying ammonifying, nitrifying and nitrogen-fixing bacteria, the conditions of treatment being such that the insoluble tricalcium phosphate of the raw mineral becomes wholly or in part converted into a form or forms, such as mono-, di-, or tetra-calcium phosphate, in which the phosphoric acid is relatively soluble and available as plant food. Aside from this decomposing action on the mineral, the nitro-bacteria present in the resultant material also exert a beneficial action on the soil upon which the material is used as a fertilizer, by increasing its available nitrogen content. The material may be made a complete fertilizer by adding potash salts thereto; or a mixture of phosphate rock and a potassiferous mineral, such as feldspar, may be acted upon in the first instance by decomposing bacteria, that is, bacteria capable of effecting decomposition, resulting in the production of a fertilizer containing both potash and phosphoric acid in available form, as well as beneficial bacteria.

It has been the practice heretofore to treat raw phosphate rock with sulfuric acid to render its contained phosphoric acid available as plant food. But this method involves considerable expense and technical difficulty. It is still more difficult and expensive, according to methods heretofore proposed, to render available the potash contained in natural minerals such as feldspar, alunite, leucite, etc. In contrast to such methods, the present invention affords a ready and comparatively inexpensive way of solubleizing or rendering available the fertilizer values locked up in many minerals, especially phosphatic and potassiferous minerals; and furthermore the resultant product is more effective and valuable as a fertilizer than the products obtained by said other methods.

In order to explain clearly the principles of the invention, the application thereof to the treatment of raw phosphate rock will be described in detail. As above pointed out, however, the invention is broad in scope and accordingly the specific explanation hereinafter given is to be understood as illustrative and not limiting.

The first requirement is a suitable material, at least partly organic in character, to serve as a carrier and nutrient medium for the bacteria to be used. Humus-like or humus-containing material, of which humus, muck, peat, etc., are typical, is most desirable for this purpose, and peat itself is peculiarly adapted, by reason of its composition and other characteristics, to give good results. In preparing it for use, the peat is thoroughly drained in the field and is then worked frequently until it is well aerated and in good productive condition. It is then collected, sun dried or artificially dried, and generally sterilized by streaming steam or dry heat to destroy its microscopical life as well as any weed seeds that may be present. By this treatment, the peat may be freed from molds and harmful bacteria. A careful grinding or sifting puts the dried peat in good condition for further treatment. If the peat available is acid in reaction, this should be corrected by the addition of alkaline or antacid substances such as well slaked lime, calcium carbonate, carbonates of pot-

ash, soda, magnesia, etc., for the reason that a slightly alkaline medium is most favorable to growth of the bacteria employed in the present process. It is also desirable to increase the nutrient properties of the peat or humus and its efficiency as a medium for bacterial growth by adding small percentages of sugar, glucose, ground sprouted barley, ground malt, extract of malt, waste products from sugar manufacture, water, or the like; and finely ground sea weed, kelp, wood ashes, or potash-bearing rock. A small amount of sulfur in the mixture also has an excellent effect; and adding an ammonium salt, such as ammonium sulfate, is particularly effective in initiating and promoting bacterial growth. Any or all of these ingredients, and others of similar character, may be used to promote bacterial activity; the choice of ingredients and the proportions thereof depending upon the character and composition of the particular lot of peat undergoing preparation, and upon the particular species of bacteria to be used.

In practice it is generally most convenient to thoroughly mix together the prepared peat or humus and the finely divided rock phosphate. To insure the best results, it is best to use phosphate rock which has been ground to fine mesh. Where the peat used is of good quality and comparatively high in organic nutrient matter, a complete mixture ready for inoculation may be made up in substantially the following proportions, per ton.

Finely ground raw phosphate rock	900	pounds
Moist sifted peat or humus	925	"
Calcium carbonate	100	"
Wood ashes	65	"
Ammonium sulfate	5	"
Extract of malt	5	"

Sufficient tap-water to allow for evaporation.

This mixture is inoculated with a pure culture, or with mixed cultures of suitable nitro-bacteria and peptonizing bacteria. The term nitro-bacteria is here used to designate generically such bacteria as ammonifying bacteria, nitrifying bacteria (nitrosomonas), nitrate bacteria, (nitrobacter), nitrogen-fixing bacteria, both symbiotic (pseudomonas radicicola) such as infest the roots of legumes, and non-symbiotic (azotobacter); also certain nitrogen-fixing bacteria such as clostridium pasteurianum, for example, and others which I have not identified by species, but whose presence, though not essential, seems to have a desirable stimulating effect upon the growth of the other bacteria above mentioned. Virulent cultures of the various bacteria required, either pure or mixed, can be prepared to best advantage by methods set forth, for example, in my prior Patents Nos. 816,856 and 865,965; and inoculation of the prepared peat and phosphate rock mixture therewith can be effected by suitably diluting the agar-agar or other original culture with water, and sprinkling it upon the mixture which is thoroughly stirred meanwhile.

In practice it is found most desirable to use mixed cultures of ammonifying, nitrifying and nitrogen-fixing (symbiotic and non-symbiotic) bacteria for inoculating the peat, as most efficient conversion of the raw phosphate rock is thereby attained, and at the same time the resultant fertilizer material is also especially desirable as a means for distributing legume bacteria and for application to the soil.

It will be seen that in the foregoing specific example the moistened humus material constitutes roughly about one-half of the mixture; and while the proportions may be varied considerably in practice, it is usually desirable to have the humus material or its equivalent, together with the other ingredients added to promote bacterial growth, constitute a large or even a major proportion of the mixture, so that the converting bacteria may flourish and multiply rapidly.

Instead of mixing the peat and phosphate rock before inoculating, either the peat or the rock may be first inoculated, and mixture effected afterward. It is often desirable to sterilize the phosphate rock also before using it.

It is of course immaterial for the purposes of this invention what may be the true explanation of the decomposing or solubleizing action of the bacteria on the raw phosphate rock in the foregoing example; but a probable explanation seems to be that the ammonifying bacteria decompose the organic nutrient material of and in the humus, forming ammonia and organic acids; and that the nitrifying or nitrite bacteria convert the ammonia into nitrous acid, which reacts with the tri-calcium phosphate of the phosphate rock to give soluble di-calcium or mono-calcium phosphate, or both, and calcium nitrite. If nitrate bacteria (nitrobacter) are present, the nitrite eventually is oxidized to nitrate.

Whatever may be the correct explanation, the fact remains that raw rock phosphate, when mixed with inoculated humus as herein described, undergoes a conversion which renders its phosphoric acid available. The rate of conversion depends upon various factors such as temperature, intensity of inoculation, etc., and is accordingly controllable within certain limits. It is practicable to ship the inoculated material to the dealer or farmer at once; but in order to insure a virulent growth and large numerical increase of the active microorganisms,

ith resultant high efficiency in the decomosing or converting action on the phoshate rock, it is much better to store the aterial for some time at a temperature avorable to bacterial development. As a ule, storage at approximately 25° C. for period of from 35 to 50 days gives an bundant growth of highly actice bacteria nd a sufficiently high degree of conversion f insoluble phosphate into available form o render the product ready for market.

As aids to nitro-bacteria in the conver-ion process of the present invention may be mployed other acid-producing bacteria, ctic acid and butyric acid bacteria, for exmple, being very efficient when used in onjunction with nitro-bacteria in the manr above set forth.

The fertilizer material obtained as decribed may be modified to give a complete rtilizer by mixing with it compounds of otash and nitrogen, for example, in suitble proportions for any desired formula.

In treating potash-bearing minerals, such s feldspar, gneissalunite and leucite, or ther minerals containing plant food in unvailable form, in accordance with the inention, the procedure is similar in general o that herein recommended for the treatent of raw phosphate rock.

What I claim is:

1. The process of preparing a fertilizer hich comprises subjecting finely divided aw phosphate rock to the decomposing acion of nitro-bacteria in the presence of an dmixed humus-like material which has een treated to improve it as a culture meium.

2. The process of preparing a fertilizer hich comprises treating peat or humus to emove acidity, adding thereto a promoter f bacterial activity, admixing finely diided phosphatic rock, and inoculating the ixture with nitro-bacteria in virulent conition to effect decomposition of the phoshate rock.

3. The process of preparing a fertilizer hich comprises inoculating humus with itro-bacteria and other acid-producing bacria, and subjecting raw phosphate rock to he decomposing action of the inoculated umus.

4. The process of preparing a fertilizer hich comprises subjecting a mineral conining plant food in unavailable form to he decomposing action of nitro-bacteria in he presence of an admixed humus-like maerial which has been treated to improve it s a culture medium.

5. The process of preparing a fertilizer hich comprises preliminarily treating huus-like material to improve it as a medium r bacteria, intimately mixing the treated aterial with a finely divided mineral containing plant food in unavailable form, and inoculating the mixture with ammonifying, nitrifying and nitrogen-fixing bacteria to render the plant food in said mineral available.

6. The process of preparing a fertilizer which comprises intimately commingling humus-like material, and finely divided phosphatic and potassiferous minerals, with ammonifying, nitrifying, and nitrogen-fixing bacteria, to effect a chemical change in said minerals.

7. The process of preparing a fertilizer which comprises growing nitro-bacteria and lactic acid bacteria in a mixture of humus and a mineral containing plant food.

8. The process of preparing a fertilizer which comprises subjecting an intimate mixture of finely divided phosphatic and potassiferous minerals with humus-like material to the decomposing action of nitro-bacteria.

9. The process of treating mineral matter to render available plant food contained therein in unavailable form which comprises subjecting such mineral matter to the decomposing action of humus-like material inoculated with nitro-bacteria.

10. The process of treating mineral matter to render available plant food contained therein in unavailable form which comprises subjecting such mineral matter to the decomposing action of humus-like material inoculated with nitro-bacteria, said humus-like material containing an added substance serving to promote bacterial activity.

11. The process of preparing a fertilizer containing available phosphoric acid which comprises mixing peat or humus, finely divided phosphate rock, and an antacid substance, and inoculating the mixture with bacteria capable of exercising a decomposing action on said phosphate rock.

12. The process of preparing a fertilizer containing available phosphoric acid which comprises mixing peat or humus, finely divided phosphate rock, a suitable antacid substance, and nutrient material, and inoculating the mixture with bacteria capable of exercising a decomposing action on said phosphate rock.

13. The process of preparing a fertilizer containing available phosphoric acid which comprises inoculating phosphate rock with selected nitro-bacteria capable of exercising a decomposing action thereon, and maintaining the inoculated rock under incubating conditions until a substantial proportion of the phosphoric acid of said rock has been rendered available for plant food.

14. The process of preparing a fertilizer which comprises inoculating phosphate rock with nitrifying bacteria and maintaining the inoculated rock at a favorable incubating

temperature until a substantial proportion of the phosphoric acid of said rock has been rendered available for plant food.

15. The process of preparing a fertilizer which comprises preparing a mixture of finely divided phosphate rock, a nutrient medium, and ammonifying, nitrifying, and nitrogen-fixing bacteria, and maintaining the inoculated mixture under incubating conditions until a substantial proportion of the phosphoric acid of said rock has been rendered available for plant food.

16. The process of preparing a fertilizer which comprises preparing a mixture consisting mainly of finely divided phosphate rock and humus-like material, said mixture also containing a small percentage of an ammonium salt and being inoculated with nitrifying bacteria, and maintaining the mixture under suitable incubating conditions to effect conversion of said phosphate rock.

17. The process of preparing a fertilizer which comprises preparing a mixture of finely divided phosphate rock and a culture medium for bacteria, said mixture being inoculated with nitro-bacteria and said culture medium constituting a relatively large proportion of the mixture, and incubating the mixture.

18. The process of preparing a fertilizer which comprises preparing a mixture of finely divided phosphate rock, humus-like material, and agents promoting bacterial growth, the mixture being inoculated with cultures of several different kinds of nitro-bacteria, and the phosphate rock constituting not more than about one-half of the mixture, and maintaining the mixture at approximately 25° C. for a period of time sufficient to effect extensive conversion of the phosphate rock.

19. A fertilizer composition comprising humus-like material inoculated with nitro-bacteria and containing an added promoter of bacterial activity, in intimate mixture with a finely divided mineral naturally containing plant food in unavailable form but modified *in situ* by said inoculated material.

20. A fertilizer composition comprising inoculated humus material intimately admixed with phosphate rock chemically modified by said inoculated humus material.

21. A fertilizer composition comprising humus material inoculated with nitrifying and nitrogen-fixing bacteria, intimately admixed with phosphate rock chemically modified by said inoculated humus material.

22. The process of preparing a fertilizer which comprises subjecting a mixture of natural phosphatic and potassiferous minerals to the decomposing action of nitro-bacteria in the presence of a food for bacteria.

23. The process of preparing a fertilizer which comprises growing nitro-bacteria and lactic acid bacteria in a mixture comprising a food for bacteria and a mineral containing plant food.

24. The process of treating mineral matter to render more available plant food contained therein, which comprises growing bacteria in a mixture comprising said mineral matter, a bacteria food, and sulfur, the bacteria employed being capable of acting in the mixture to render the contained plant food more available.

25. The process of treating mineral matter to render more available plant food contained therein, which comprises growing nitro-bacteria in a mixture comprising said mineral matter, a bacteria food, and sulfur.

26. The process of treating phosphatic mineral matter to render available phosphoric acid contained therein, which comprises growing nitro-bacteria in a mixture comprising said mineral matter, a nutrient medium, and sulfur.

27. The process of treating phosphate rock which comprises subjecting phosphate rock to the decomposing action of a slightly alkaline culture medium containing sulfur and inoculated with suitable nitro-bacteria.

28. The process of treating mineral matter to render available plant food contained therein, which comprises growing nitro-bacteria in a mixture comprising such mineral matter and a malt nutrient.

29. The process of treating mineral matter to render available plant food contained therein, which comprises growing nitro-bacteria in a mixture comprising such mineral matter and ground sprouted barley.

30. A fertilizer composition comprising fertilizer-containing mineral matter mixed with nutrient and carrier material containing sulfur and carrying bacteria capable of acting in the mixture to render more available fertilizer contained in said mineral matter.

31. A fertilizer composition comprising phosphate rock, mixed with nutrient and carrier material containing sulfur and carrying bacteria capable of acting in the mixture to render the phosphoric acid content thereof more available.

32. A fertilizer composition comprising phosphate rock, mixed with nutrient and carrier material containing sulfur and carrying nitrifying bacteria.

33. A fertilizer composition comprising phosphate rock, mixed with nutrient and carrier material containing sulfur and carrying ammonifying, nitrifying and nitrogen-fixing bacteria.

34. The process of preparing a fertilizer material which comprises preparing a mixture of a finely-divided phosphatic mineral with selected nitro-bacteria and a food for

aid bacteria, and maintaining the mixture
t a temperature not substantially less than
bout 25° C. to promote development of said
acteria and thereby to render the phos-
horic acid content of said mineral more
vailable.

35. The process of preparing a fertilizer
aterial which comprises subjecting mate-
rial containing compounds of both phos-
phorus and potassium chiefly in relatively
unavailable form to the decomposing action
of selected bacteria in the presence of a food
for bacteria.

In testimony whereof I hereunto affix my signature.

GEORGE H. EARP-THOMAS.

UNITED STATES PATENT OFFICE.

THOMAS FRANKLIN MANNS, OF NEWARK, DELAWARE.

PROCESS OF REINFORCING, BACTERIZING, AND COMPOSTING ORGANIC MATERIAL FOR USE AS INOCULATORS, IMPROVERS, AND FERTILIZER OF SOILS.

1,320,701. Specification of Letters Patent. Patented Nov. 4, 1919.

No Drawing. Application filed October 18, 1915. Serial No. 56,040.

To all whom it may concern:

Be it known that I, THOMAS F. MANNS, a citizen of the United State, residing at Newark, in the county of New Castle and State of Delaware, have invented certain new and useful Improvements in Processes of Reinforcing, Bacterizing, and Composting Organic Materials for Use as Inoculators, Improvers, and Fertilizer of Soils, of which the following is a specification.

This invention relates to a process of reinforcing, bacterizing, and composting organic material, such as peat, muck, manure, and the like for use as a soil inoculator, soil mellower, plant stimulator and fertilizer.

I have discovered that peat, muck, manure, weed compost or other organic material can be transformed into a fertilizer containing an accumulation of nitrate nitrogen by bacterial action and a humus content having a high stimulative influence on plant life and possessing great value as a plant food, a soil improver and fertilizer, by reinforcing or adding to the organic matter, phosphorus in the form of basic slag, or potassium, sodium or calcium phosphate, either the neutral or acid forms, but preferably the commercial calcium acid phosphate, and then by the addition of a base, either wood ashes, basic slag, potassium carbonate, calcium carbonate or hydrate, but preferably calcium hydrate with certain amounts of wood ashes, basic slag and potassium carbonate, to neutralize the mixture, and then by bacterizing and composting.

The practice of my process will be described in connection with the treatment of peat but it is to be understood that it is equally applicable to other organic materials.

In the practical application of my process to the treatment of peat there are produced two important products each having a high commercial value. The first product, which I shall refer to herein as the bacterial compost, bacterizer or inoculator, is a rich compost containing all the known beneficial soil bacteria. This product, when partially air dried without destroying the beneficial organisms contained therein, is used to abundantly inoculate the second product which is peat or other organic matter sufficiently dried, and reinforced with proper mineral and organic compounds. The latter product is sent out commercially in a partially dried state which, when moistened in the soil to which it is applied, will set up bacterial action from the thorough inoculation. Soil inoculation and plant stimulation will result bringing high crop response.

In the practical application of my process, in the production of the first product, viz., the bacterial compost or bacterizer, I add to peat or other organic matter, a phosphate, either the neutral or acid phosphates of sodium, potassium or calcium or Thomas slag, but preferably a sixteen per cent. (16%) commercial calcium acid phosphate, and a sufficient quantity of some alkali metal or alkaline earth metal compound to neutralize the product, such as basic slag, calcium, sodium, or potassium carbonate, or the hydrates of these alkalis or alkaline earths, but preferably a mixture of calcium carbonate, calcium hydrate, basic slag and wood ashes, to which entire mixture is added a quantity of a carbohydrate such as molasses or sugar and protein matter such as dried blood, tankage, cottonseed meal or similar organic matter. At the time of mixing, the proper bacterial flora is supplied through a mixture of soils known to contain an abundance of the desired organisms. In case the soils used are deficient in any of the desired legume organisms pure cultures may be used.

I have found that a satisfactory compost will result from a mixture of peat, calcium phosphate or calcium acid phosphate, and calcium carbonate, or a mixture of substantially equal parts of calcium carbonate and calcium hydrate and inoculated soils in the following proportions:

Peat	1 ton.
Commercial calcium acid phosphate	50 lbs.
Calcium hydrate	50 lbs.
Soils, well inoculated	25 lbs.

In my experiments with my process I have found it advantageous to add the alkali metal or alkaline earth metal salts in sufficient quantity to overcome the acidity of the acid phosphate added and the peat or other organic material under treatment. I have also found that improved results may be obtained by the use of additional mineral materials, such for example as potassium

salts, notably potassium chlorid, potassium sulfate and potassium carbonate, wood ashes and Thomas slag. I have also found it advantageous to add to the mixture of peat and mineral matter a liberal amount of molasses or sugar, and dried blood, preferably 30 pounds of the former and 10 pounds of the latter to each ton of organic matter.

I have obtained particularly advantageous results in my bacterial compost or bacterizer, from the use of peat, calcium, acid phosphate (commercial) calcium phosphate (commercially known as "floats"), calcium hydrate, calcium carbonate, wood ashes, Thomas slag, molasses and dried blood, and properly inoculated soil in the following proportions by weight:

Peat	1 ton.
Calcium acid phosphate	5 lbs.
Rock phosphate (known commercially as "floats")	50 lbs.
Calcium hydrate	20 lbs.
Calcium carbonate (ground limestone)	100 lbs.
Wood ashes	200 lbs.
Thomas slag	10 lbs.
Dried blood	10 lbs.
Molasses (or cane sugar 25 lbs)	30 lbs.
Well inoculated soil	25 lbs.

The peculiar efficiency of this bacterial compost is believed to be due primarily to the well balanced mixture containing all the ingredients or compounds needed for the intended purpose, such ingredients being properly proportioned to give rapid growth to each of the groups of beneficial soil bacteria. I obtain a further advantage from the inoculating material which I employ. This inoculating material is preferably selected soils from widely different areas of the United States, definitely proven for their efficiency in regard to certain physiological activity, (for example, nitrogen fixation; cellulose destruction in which the cellulose becomes food for further bacterial action; the changing of organic nitrogen compounds to ammoniacal and nitric nitrogen). Some of the sources of this inoculating material are as follows: For azotobacter, that is the non-symbiotic nitrogen fixing organisms, and nitrosomonas and nitrobacter, the former oxidizing ammonia to nitrite, and the latter completing the oxidation to nitrate, I use the active azo or nitrogen fixing soils of Colorado and North Dakota, those soils bordering on the so called "niter-spots". For introducing the cellulose destroyers *B. rossica* and related bacteria I use a mixture of rapidly composting manure or a soil known to be high in these organisms. For introducing the ammonifiers I use a soil of high ammonifying efficiency as proven by the destruction of paper, inserted therein. For introducing the legume bacteria I use a well inoculated soil upon which has grown the particular legume for which the inoculation is introduced. Recent scientific knowledge (Bulletin 184, Kentucky Aug. 1914) and (*Centralblatt für Bakt.* 40 Bd. No. 11-13—M... 1914) has shown that there are only ... species of *B. radicicola* and six of these species or physiological groups cover the inoculation of the most important agricultural legumes. So in practice I employ thoroughly inoculated soil from a legume of each of the six groups, thereby introducing inoculation for all the important agricultural legumes in the one compost. In practice this material has been found superior to the so called "pure culture legume inoculating material." In practice, when it is difficult to obtain the soil well inoculated for the particular legume, I use a pure culture for the same purpose, though the soil gives superior results. In practice I find this product to give thorough inoculation to the following legumes, viz: alfalfa, sweet clover (white), sweet clover (yellow), black medic or yellow trefoil, bur clover, red clover, mammoth clover, alsike clover, white clover, soy beans, cow peas, sweet peas, spring vetch, garden peas, Canada field peas, lima beans, navy beans, velvet beans and the various other legumes commonly grown as soil improvers.

In practice the peat or other organic matter to be composted, the reinforcing and balancing minerals and compounds (except ing the calcium hydrate which is added to the well diluted mixture of molasses), the dried blood and the inoculated soil which in the preferred embodiment of my invention contains all the beneficial bacteria herein before specified, are thoroughly mixed by hand using shovels, or the whole mixture is passed through any efficient fertilizer mixer; the material is then spread out and the solution of molasses containing the lime hydrate is evenly sprayed on, then the material is spread out to a depth of preferably from two to four feet and moistened down by a Skinner irrigation system or other means of watering and allowed to undergo aerobic composting for two weeks to three months. The product is examined from time to time, and when the bacterial flora has reached the growth required the product is ready for use for field, lawn or garden inoculation, for legume inoculation and for inoculating the commercially reinforced and balanced peat. The product may be used as a soil in green houses, conservatories, or homes. Also in fields as a general fertilizer, soil inoculator and soil improver.

In practice the composting operation may be advantageously concluded when *B. radicicola* is found to be present to the

umber of approximately 200 billions per ound of the moist compost. It has been ound in practice that when *B. radicicola* as developed to the extent indicated, the zotobacters are present in approximately 0 billions per pound of moist compost and itrosomonas and nitrobacter are present in pproximately 25 billions. The ammoni- ers and cellulose destroyers approach the atter numbers viz., about 25 billions per ound of moist compost.

It has been found that the addition of ineral materials herein before referred to, articularly in the proportions specified, roduce a balanced material and a bacterial edium particularly suitable for the growth f a well balanced and efficient bacterial ora in which each of the beneficial groups eep pace with one another in an environ- ent conducive to symbiotic relationship.

It has been found in practice that in two eeks of composting a considerable quantity f nitrate nitrogen is formed, equivalent or- inarily to over one pound of nitrates to ach ton of compost. Likewise I have found n practice an accumulation of nitrogen by he activity of the nitrogen fixing organ- sms, equivalent ordinarily to about one ound of high protein matter per ton of ompost in one month. The addition of he alkali or alkaline earth metal phosphate nd the other ingredients mentioned in the articular mixture above set forth produces particularly favorable condition in the edium or material for the rapid and ex- nsive development of *B. radicicola*, *zotobacter chroococcum* and related bac- ria, nitrosomonas and nitrobacter, all rganisms of great importance to agricul- ure, in a desired and particularly advan- ageous relationship as to the development f each.

In the practice of my process I have ound that there is a close symbiosis be- ween *B. radicicola* and *Azotobacter chroo- occum* and especially that the gummy or elatinous exude of *B. radicicola* is a fa- orable medium in which to develop.

My second product consists in reinforced balanced peat that has been inoculated bacterized with the bacterial compost or acterizer. This second material or rein- orced and inoculated or bac rized peat is ot composted previous to placing it on the arket. The reinforcement or addition of ineral and or anic compounds make it at nce a valuable fertilizer and a favorable edium for bacterial development, as soon it is moistened. In the practical appli- ation of my process in producing this prod- t, the peat is partially dried, preferably movement over a rotary drier, it is then ound fine, and the reinforcing material dded. This material is added in sufficient uantity to make the product an immedi- ately responsive fertilizer and at the same time a very favorable medium or food for the rapid development of the beneficial bacteria which have been applied. My second mixture is preferably made in approxi- mately the following proportions:

Peat	1800 lbs.
High grade tankage	40 lbs.
Floats (ground rock phosphate)	60 lbs.
Acid phosphate (commercial 16% acid phosphate)	20 lbs.
Wood ashes (8% potash)	100 lbs.
Lime hydrate	20 lbs.
Nitrate of soda (18% nitrate)	10 lbs.
Granulated sugar	3 to 10 lbs.
Bacterial compost or bacterizer	50 lbs.

In practical application this product is found to give to plants a high fertilizing response. It is very effective in mellowing heavy clays and retaining moisture and holding together sands. It is especially efficient on lawn and turfs. It is also highly stimulating to field crops, such as wheat, other grains and grasses and is very valuable as a truck crop stimulator. It has been found in practice to give thorough inoculation to all soils to which applied and all agricultural legumes take a thoroughly satisfactory inoculation. This is an advantage which it is believed is not possessed by any other single material intended for the same purpose.

The cost of the ingredients employed in my process and the inexpensiveness of the operation makes it possible in practice to sell this product commercially at such a low price per ton that it may be used freely as a field fertilizer as well as a soil inoculator and a soil improver.

It is to be understood that the present invention is not restricted to the illustrative examples herein before set forth, it being understood that known equivalents of the materials may be used, nor to the proportions of the ingredients specified nor to the exact details of procedure set forth, it being understood that these may be varied without departing from the spirit of my invention or the scope of the subjoined claims.

Having thus described the invention, I claim:—

1. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in mixing peat-like organic material of the character specified, a phosphate, a base in sufficient amount to produce a substantially neutral mixture, protein matter and carbo-hydrate matter, inoculating the mixture with azotobacters, *B. radicicola*, nitrobacters, and *B. rossica*, and composting the mixture.

2. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in adding to peat-like organic material of the character specified, a phosphate, a base in sufficient amount to produce a substantially neutral mixture, dried blood and sugar, inoculating the mixture with azotobacters, *B. radicicola*, nitrobacters, and *B. rossica*, and composting the mixture.

3. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in thoroughly mixing unsterilized peat, a phosphate, a base in sufficient amount to produce a substantially neutral mixture, dried blood and sugar, inoculating the mixture by the addition thereto of a mixture of soils well inoculated with azotobacters, *B. radicicola*, nitrobacters, and *B. rossica*, and composting the mixture.

4. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in thoroughly mixing peat, calcium acid phosphate, calcium hydrate, sugar and dried blood in approximately the following proportions: peat 1 ton, calcium acid phosphate 50 lbs., calcium hydrate 50 lbs., sugar 25 lbs., and dried blood 10 lbs., inoculating the mixture with azotobacters, *B. radicicola*, nitrosomonas, nitrobacters, and *B. rossica*, and composting the mixture.

5. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in thoroughly mixing peat, calcium acid phosphate, rock phosphate, calcium hydrate, calcium carbonate, wood ashes, Thomas slag, sugar and dried blood, inoculating the mixture with azotobacters, *B. radicicola*, nitrosomonas, nitrobacters, and *B. rossica*, and composting the mixture.

6. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in thoroughly mixing peat and the following materials in substantially the proportions specified: to each ton of peat, calcium acid phosphate 5 lbs., rock phosphate 50 lbs., calcium hydrate 20 lbs., calcium carbonate 100 lbs., wood ashes 200 lbs., Thomas slag 10 lbs., sugar 25 lbs., and dried blood 10 lbs., inoculating the mixture with azotobacters, *B. radicicola*, nitrosomonas, nitrobacters, and *B. rossica*, and composting the mixture.

7. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in adding to peat-like organic material of the character specified, a phosphate, a base in sufficient amount to produce a substantially neutral mixture, carbohydrate matter and protein matter, inoculating the mixture with organisms of a plurality of groups of beneficial soil bacteria, composting the mixture, and adding a portion of the resulting product to a mixture of peat-like organic material of the character specified, rock phosphate, calcium acid phosphate, wood ashes, calcium hydrate, sodium nitrate, carbo-hydrate and protein matter.

8. The herein described process of producing a bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes which consists in thoroughly mixing peat and the following materials in substantially the proportions specified; to one ton of peat, calcium acid phosphate 5 lbs., rock phosphate 50 lbs., calcium hydrate 20 lbs., calcium carbonate 100 lbs., wood ashes 200 lbs., Thomas slag 10 lbs., sugar 25 lbs., and dried blood 10 lbs., inoculating the mixture by adding a mixture of soils well inoculated with azotobacters, *B. radicicola*, nitrosomonas nitrobacters, and *B. rossica*, composting the mixture and adding a portion of the product to a mixture of peat with the following materials, in substantially the proportions specified: to 1800 lbs. of peat, rock phosphate 60 lbs., commercial calcium acid phosphate 20 lbs., sodium nitrate 10 lbs., granulated sugar, 3 to 10 lbs., high grade tankage 40 lbs.

9. The herein described bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes, containing peat, a phosphate, a base in sufficient amount to produce a substantially neutral mixture, protein matter and carbo-hydrate matter, such material being thoroughly inoculated with azotobacters *B. radicicola*, nitrosomonas, nitrobacters and *B. rossica*.

10. The herein described bacterial compost or bacterizer containing organisms necessary for the inoculation of the different agricultural legumes, comprising peat, commercial calcium acid phosphate, calcium hydrate, sugar and dried blood in substantially the following proportions: peat, one ton, calcium acid phosphate 50 lbs., calcium hydrate 20 lbs., sugar 25 lbs., dried blood 10 lbs., said material being thoroughly inoculated with azotobacters, *B. radicicola*, nitrosomonas, nitrobacters, and *B. rossica*.

11. The herein described bacterial compost or bacterizer, containing organisms necessary for the inoculation of the different

agricultural legumes, containing the following materials in substantially the proportions specified: peat 1 ton, commercial calcium acid phosphate 5 lbs., rock phosphate 50 lbs., calcium hydrate 20 lbs., calcium carbonate 100 lbs., wood ashes 200 lbs., Thomas slag 10 lbs., sugar 25 lbs., protein matter 10 lbs., the material being well inoculated with azotobacters, *B. radicicola*, nitrosomonas, nitrobacters, and *B. rossica*.

In testimony whereof I affix my signature in presence of two witnesses.

THOMAS FRANKLIN MANNS.

Witnesses:

Mrs. ALYS MANNS,

M. R. APSLEY.

1231 UNITED STATES OF AMERICA
SOUTHERN DISTRICT OF ILLINOIS } ss:
SOUTHERN DIVISION

I, G. W. Schwaner, Clerk of the District Court of the United States in and for the Southern District of Illinois and keeper of the records and the Seal thereof, do hereby certify the foregoing to be a true and complete transcript of the proceedings had of record and on file in the cause entitled KALO INOCULANT COMPANY, A CORPORATION *vs.* FUNK BROTHERS SEED COMPANY, A CORPORATION, CIVIL ACTION NUMBER Q-62, made in accordance with the Stipulated Designation of Record on Appeal, as fully as the same appear from the original files and records thereof now in my office remaining. And I further certify that in accordance with the Stipulation of counsel approved by the court, the original transcript of the Record of Proceedings at the Trial has been incorporated in the said transcript of record on appeal.

IN TESTIMONY WHEREOF, I have hereunto subscribed my name and affixed the Seal of the aforesaid Court, at Quincy, this 1st day of March, A. D. 1946.

G. W. Schwaner Clerk.

(Seal)

UNITED STATES CIRCUIT COURT OF APPEALS

For the Seventh Circuit.

I, Kenneth J. Carrick, Clerk of the United States Circuit Court of Appeals for the Seventh Circuit, do hereby certify that the foregoing printed pages contain a true copy of Volume 2 of the Printed Record, being one of 2 Volumes of said Printed Record filed on October 19, 1946, in:

Cause No. 9040-41.

Kalo Inoculant Company,
Plaintiff-Appellant,

vs.

Funk Brothers Seed Company,
Defendant-Appellee,

and

Kalo Inoculant Company,
Plaintiff-Appellee,

vs.

Funk Brothers Seed Company,
Defendant-Appellant,

as the same remains upon the files and records of the United States Circuit Court of Appeals for the Seventh Circuit.

In Testimony Whereof I hereunto subscribe my name and affix the seal of said United States Circuit Court of Appeals for the Seventh Circuit, at the City of Chicago, this 1st day of August, A. D. 1947.

Kenneth J. Carrick,
(Seal) *Clerk of the United States Circuit Court of Appeals for the Seventh Circuit.*

At a regular term of the United States Circuit Court of Appeals for the Seventh Circuit, held in the City of Chicago, and begun on the second day of October, in the year of our Lord one thousand nine hundred and forty-five, and of our Independence the one hundred and seventieth.

Kalo Inoculant Company,
Plaintiff-Appellant,
9040 *vs.*
Funk Brothers Seed Company,
Defendant-Appellee.

Kalo Inoculant Company,
Plaintiff-Appellee,
9041 *vs.*
Funk Brothers Seed Company,
Defendant-Appellant.

Appeals from the District Court of the United States for the Southern District of Illinois, Southern Division.

And afterwards, to-wit: On the eighth day of May, 1947, the following proceedings were had and entered of record, to-wit:

Thursday, May 8, 1947.

Court met pursuant to adjournment.

Before:

Hon. Evan A. Evans, Circuit Judge.
Hon. J. Earl Major, Circuit Judge.
Hon. Walter C. Lindley, District Judge.

Kalo Inoculant Company, *Plaintiff-Appellant,* vs. Funk Brothers Seed Company, *Defendant-Appellee.* 9040	Appeals from the District Court of the United States for the Southern District of Illinois, Southern Division.
Kalo Inoculant Company, *Plaintiff-Appellee,* vs. Funk Brothers Seed Company, *Defendant-Appellant.* 9041	

Now this day come the parties by their counsel, and this cause comes on to be heard on the transcript of the record and the briefs of counsel, and on oral argument by Mr. J. Bernhard Theiss for the Kalo Inoculant Company, and by Mr. H. A. Toulmin, Jr. for the Funk Brothers Seed Company, and the Court takes this matter under advisement.

And afterwards, to-wit: On the twelfth day of June, 1947, there was filed in the office of the Clerk of this Court, the Opinion of the Court, which said opinion is in the words and figures following, to-wit:

IN THE UNITED STATES CIRCUIT COURT OF APPEALS

For the Seventh Circuit.

October Term, 1946, April Session, 1947.

KALO INOCULANT COMPANY, *Plaintiff-Appellant,* vs. FUNK BROTHERS SEED COMPANY, *Defendant-Appellee.* — No. 9040.	Appeals from the District Court of the United States for the Southern District of Illinois, Southern Division.
KALO INOCULANT COMPANY, *Plaintiff-Appellee,* vs. FUNK BROTHERS SEED COMPANY, *Defendant-Appellant.* — No. 9041.	

June 12, 1947.

Before EVANS, MAJOR, *Circuit Judges,* and LINDLEY, *District Judge.*

LINDLEY, *District Judge.* Plaintiff, manufacturer and vendor of bacterial inoculant for leguminous plants, brought suit for infringement of patent to Bond No. 2,200,532, issued May 14, 1940, on application filed August 24, 1938, and assigned to plaintiff, against defendant, wholesale and retail dealer in certain leguminous inoculants, alleged to be infringing products manufactured by The Agricultural Laboratories, Inc., of Columbus, Ohio, who assumed control and direction of the defense. The District Court adjudged the claims upon which plaintiff relied invalid for want of invention, finding, however, that, if they were valid, defendant infringed. The court dismissed defendant's counter-

claim for declaratory judgment of invalidity of all claims of the patents, including those in suit. Plaintiff appeals from the judgment of invalidity and defendant from the dismissal of its cross-complaint.

The essential facts, as reflected by the court's findings, are not largely in dispute. The patent contains 14 claims. Those in suit 1, 3, 4, 5, 6, 7, 8, 13 and 14 and 2, not in suit, are product claims.* The others, 9, 10, 11 and 12, are process or method claims. The patent is entitled "Bacterial Inoculant for Leguminous Plants." Its descriptive matter includes recital of scientific facts, then known to scientists delving in the art, and prescribes certain old conventional tests necessary in successful production of bacteria for leguminous plants. The claims cover a composite culture in which are included a plurality of species of bacteria belonging to the general Rhizobium genus, carried in a conventional base. The claim of invention rests primarily upon the fact that each separate culture of the several different species included in the composite inoculant is unaffected by the presence of others in its ability to fix nitrogen in the leguminous plant for which it is specific. It is undisputed that bacteria of this genus promote nitrogen fixation in leguminous plants and are found in nature. The precise mechanism of nitrogen fixation is not understood, but it has been well known for many years that it can be achieved only when the plant grows in symbiotic relationship with certain bacteria of the genus. Leguminous plants capable of such relationship with Rhizobia include peas, beans, alfalfa, red clover, white clover, vetch, lupines and others. When nitrogen is collected or fixed, it appears in the form of nodules on the roots of the plants; the bacteria infect the roots, causing formation of the nodules in which nitrogen fixation occurs. Scientists have known for a long time that Rhizobium bacteria promote nitrogen fixation when a certain species or strain of the same is applied in symbiotic relationship to the leguminous plant of the cross inoculation group to which the particular species or strain is specific. Manufacturers of inoculants have, for a long time, taken these bacteria as found in nature, where they perform this nitrogen fixation, and, by well known

*Claim 4, we think, is typical of this group. It reads: An inoculant for leguminous plants comprising a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus Rhizobium, said strains being unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific.

laboratory methods of culture, increased their number, changing in no way their shape, form or characteristics, physiologically or otherwise, placed them in a powder or liquid base, as a carrying medium, and sold them for use by agriculturists in the inoculation of leguminous plants. Bond claims to be the first successfully to combine different species of the genus in one composite product so that each species will continue to perform its functions when brought into its proper symbiotic relationship. In other words, a specific species of the genus Rhizobium, functioning with its corresponding species of legume in an inoculant for its specific species of legume had, as we have said, been successfully produced and used with desirable results. As to the facts thus far related, we think there is no substantial dispute.

Bond does not claim to have invented any new species of Rhizobium bacteria but, as the District Court found, he discovered that among the bacteria found widely spread in nature there exist not only mutually inhibitive species but also mutually noninhibitive strains which can be selected and isolated and, when so selected, isolated and combined, continue to perform the same functions that they do in nature. The method of culture of single strains of bacteria described in Bond's patent and his method of placing bacteria in a carrying medium or base were well known in the art. So, too, was the proper character of the base or carrying medium. In fact, Bond's claim of invention arises from his averment of discovery that, though in the bacteria genus Rhizobium some species, when combined with others, had an injurious or adverse effect upon each other, when properly selected and combined, certain of them were noninhibitive of each other. This inhibitive character of certain species was already known to scientists, but, he added to that knowledge his own discovery, as a result of empirical tests, that there are also strains or species of bacteria involved which are noninhibitive of others, in other words, that there are noninhibitive cooperative strains as well as inhibitive, destructive strains. Thus he said: "It has heretofore been considered impractical to prepare a composite inoculant containing organisms which will cause nodulation on more than one of the cross inoculation groups. This has not been done because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic

nitrogen fixation by the plant and the organism was inhibited or even prevented." His teaching was that he could, by conventional tests, delete the inhibitive strains and retain the noninhibitive ones and that these, when combined, could be put into a composite inoculant where the same desirable results could be achieved by each appropriate species of bacteria as would be accomplished if only a single species of bacteria were included in the inoculant. Upon this we think the question of invention hinges.

The District Judge thought invention was not achieved, saying that he found the claims invalid because they did not involve invention or discovery of any new or useful art. This, he said, he did "with reluctance," as he was impressed with the very "great diligence and ingenuity" of Bond and with the results he had achieved. But, though Bond's work was not to be minimized, the Judge believed it could not be classified under any subject defined as patentable by the Congressional Act. However, he made a specific finding that Bond did discover that there exist in nature noninhibitive strains and that these noninhibitive strains may be selected, isolated and combined and still perform satisfactorily their ordinary desirable functions. Upon this finding there was controverted evidence, but our examination of the record is convincing that that supporting the finding is rather conclusive and that there is no justification for us to declare the District Court in error in making it. Consequently the specific question which now confronts us is whether, upon the finding of fact, the court erred in concluding that, under the law, Bond made nothing patentable.

We have previously said that Bond recognized everything that had been done by scientists delving in this art prior to his application. He knew that bacteriologists were well informed as to the conventional tests and the essentials of development of a bacterial culture and possessed all the knowledge necessary to successful isolation and culture of bacteria. He recognized that prior delvers in the art were aware of proper *media* for lodgment and distribution of cultures fully developed and ready for commercial use. He made no claim to have discovered in nature any species of bacteria not previously known. He knew that the different species properly suitable for the several functions to be performed had been thought to be mutually inhibitive. He did discover the fact, as the court found,

that there are strains proper for the desired purpose of combination which possess no antagonistic characteristics which, when combined, do not tend to suppress the growth of any of other species, all of which, when combined, develop in wholly noninhibited, unimpeded growth. He emphasized the fact that composite inoculants had not been successful previously and that this was generally but erroneously thought to be because the various strains when combined always inhibited each other.

This, he said, was the status of the art when in June, 1937, he first discussed with the president of his company the possibility of the manufacture of a practical composite or mixed leguminous culture. He was familiar at that time with adverse opinion of authorities in the field in regard to mixed cultures. During that summer he attended the University of Wisconsin and there investigated the literature in the library of the College of Agriculture. In his research he found adverse references to the use of mixed cultures containing more than one species of leguminous organisms and learned that it had been publicly reported that when two or more different species were mixed, they had a definite antagonistic effect upon each other which reduced their ability to benefit the host plant. He testified that he found nothing to indicate that anyone had ever thought that there were in existence strains or species in which inhibition did not exist. The known teaching, so far as he could discover at that time, was that a species of the genus might be "very good when used alone, * * * be very beneficial, * * * be a highly efficient strain, but, when mixed with other strains would become less efficient and produce a lower yield than the same strain when used alone." The so-called Bible of the inoculant art, "Root Nodule Bacteria and Leguminous Plants," by Fred, Baldwin and McCoy, University of Wisconsin Studies in Science, Number 5, 1932, reported that composite cultures "offer advantages and many disadvantages" and that "it is desirable to have only the one type of organism in the culture."

Bearing in mind that the District Court found specifically that Bond discovered that there are noninhibitive strains of the proper species of bacteria as well as inhibitive species and that Bond had been greatly diligent and ingenious, it becomes obvious, we think, that he made an inventive step forward in the art of production and commercial distribu-

tion of composite inoculants. It was desirable, as he pointed out in his patent, that farmers should have one composite inoculant which could be used in producing their different crops. In such case it would be unnecessary for dealers to keep in inventory and for the farmer to buy, preserve and maintain several different inoculants. His company was hopeful that he could manage to produce such a satisfactory composite inoculant. His problem was to find a method by which he might get away from the inhibitive, antagonistic, impeding characteristics of the different strains of bacteria when combined and, at the same time, produce a composite that would contain and maintain the proper number of live and active bacteria of each species throughout the useful life of the product. This problem had not been solved by any one and it apparently daunted Bond, for, at first, it appeared insoluble. However, in the course of continued probing, he made the discovery which nobody, prior to that time, had uncovered, namely, that there exist in nature certain strains of nodule bacteria which do not assert an inhibitive and antagonistic effect on each other and that such mutual noninhibitive strains can, by well known methods, be selected and isolated for use in a mixed culture so that the final composite product will contain, throughout its desirable life, a sufficient number of live and active bacteria of each species, lodging them in a base material substantially free of nutrients. His scientific investigation led to a proper solution, which we think the evidence shows had baffled the workers in the prior art because of their mistaken assumption of supposedly universal inhibitive effect encountered in mixed cultures. Thus, in making one of his tests, Bond found that one of the proper strains of soy bean bacteria, "Rhizobium Japonicum," was found to produce 34.8% more nitrogen when employed alone on soy bean seed than when employed on the same seed in the presence of clover bacteria and alfalfa bacteria (Rhizobium trifolii and Rhizobium meliloti). He found also that a strain of clover bacteria would produce 119% more nitrogen when employed alone than when employed in the presence of soy bean bacteria and alfalfa bacteria. Inhibitive effect was the restraining factor preventing a successful commercial composite inoculant and this factor he taught the art to avoid by discovering that there are noninhibitive bacteria and teaching that they can be successfully employed, when combined, without in-

jury to any of the other constituent species. Thus he says in his patent: "The composite culture may be prepared by selecting strains of the desired species of the Rhizobia which will produce optimum beneficial results to the host plant and will not be adversely affected by each other." And, that he taught how this might be done is illustrated by this language: "* * * Each of the strains is tested separately with its own host plant in order to select out those which are most effective in respect to the development of the host plant and the quantity of nitrogen fixed thereby. * * * The cultures showing the best results in association with the host plant may then be selected and the various strains of the different species may be mixed together in order to determine if the nitrogen-fixing ability of one species is adversely affected by the mere presence of another, as has been previously thought to be the case. Thus, it has been previously considered that the nitrogen-fixing ability of Rhizobium trifolii in symbiotic relationship with red clover is markedly inhibited if strains of Rhizobium meliloti, Rhizobium japonicum, or other species of the Rhizobia were present within the same inoculant. It has been found, however, that, by a suitable process of selection of strains in the manner indicated above, a composite culture may be obtained which may be used to effect a plurality of cross-inoculation groups with optimum results."

For convenience sake, he designated the noninhibitive strains as "alpha" organisms and said that he had discovered no method by which they might be differentiated from the mutually inhibitive group of Rhizobia other than by making actual nitroger fixation tests by inoculating the various host plants with the proposed mixture of the Rhizobia. But by "alpha" strains he did not mean that he had discovered a new bacteria but merely that certain existing bacteria are noninhibitive.

The mistake of the District Court, we think, lay in its conclusion that this did not amount to patentable invention within the meaning of the statute. Though the court recognized the value of Bond's work, it thought that he had merely discovered a law of nature, whereas in fact the evidence is clear that what he discovered was that certain existing bacteria do not possess the mutually inhibitive characteristics which had previously prevented a successful commercial composite inoculant and that those unin-

hibitive species may be successfully combined. It was this contribution of noninhibitive strains which successfully combine that brought about a new patentable composition. This was application of scientific knowledge to things existing in nature and the utilization of them in a desirable composite product which had not been previously achieved but which he did achieve and of which the public now has the benefit.

We think this is clearly within the decisive definitions of patentable invention. It is in accord with the decision of this court in *Dennis et al.* v. *Pitner et al.*, 106 F. 2d 142 (CCA7) and that of the Supreme Court in *Mackay Radio & Tel. Co.* v. *Radio Corp.*, 306 U. S. 86. See also *Union Carbide Co.* v. *American Carbide Co.*, 181 F. 104; *J. E. Baker Co.* v. *Kennedy Refractories Co.*, 253 F. 739 (CCA3). Bond taught the method by which the composite is to be made and claims the product composed by that method, namely, a composite inoculant, comprised of different species of bacteria in sufficient numbers of each species. The noninhibitive property of bacteria was not previously known and when Bond discovered it and taught a successful composition of noninhibitive strains in combination of elements in one inoculant operating successfully on several different groups of legumes, he did much more than discover a law of nature. He made a new and different composition, one contributing utility and economy to the manufacture and distribution of commercial inoculants. As this court said, in *Dennis et al.* v. *Pitner et al.*, *supra*, the discovery of a natural phenomenon, or of a quality or attribute of a well known article and application of that quality in a successful combination which is of value to mankind, is entitled to patent protection. We adhere to that pronouncement and we think Bond achieved that end.

Defendant has directed our attention to certain prior art patents, publications and uses, all of which we have studiously examined. We might discuss them at length, but we think it sufficient to say that the sum total of all of the prior teaching is that mixed inoculants had been in existence before but that they had been composed by hit and run methods without regard to whether the various species included in the composite worked adversely to one another or inhibited one another in their respective growths. Some of them may have been successful, because, by mere chance, only noninhibitive species were included. Some of them

must have been wholly useless when they included only inhibitive species, and the result must have been far from satisfactory when they included both inhibitive and non-inhibitive species. Nothing in the art suggests the slightest conception of Bond's solution of the problem.

Defendant insists also, as it did in the District Court, that the patent is either the invention of Lusk or the joint invention of Lusk and Bond. The District Court found that, though the general idea of the desirability of a composite culture was suggested by Lusk, the discovery of the concrete solution of the problem was the act of Bond and that the invention or discovery, if any, was his sole act. We agree with the court that, under the evidence, all that Lusk did was to suggest that it would be highly desirable for his company to have a composite inoculant such as Bond later invented; that Lusk had no conception of what the problem really involved or of how to solve it and that all of the investigation, research and thought leading to its solution was the work of the patentee. Certainly it can not be said that, upon the evidence in this record, this court is justified in setting aside the finding of the District Court.

Defendant also contends, as it did in the District Court, that the plaintiff misrepresented his application to the Patent Office and, therefore, does not come into court with clean hands. We think there is no evidence justifying a finding to this effect, and again we find that the court made a specific finding in this respect to the effect that Bond believed in the existence of the inhibitive effect of various species at the time he instituted his research and acted in good faith when, in his application for the patent in suit, he said that it had previously been considered impractical to prepare a composite inoculant containing organisms which would produce nodulation on more than one of the cross inoculation groups because it was generally believed that one species produced an inhibitory effect on another species within the same culture, whereby symbiotic nitrogen fixation by the plant and the organism was inhibited or even prevented and that plaintiff was not guilty of the charge of unclean hands. We have examined the evidence of what transpired in the Patent Office. We find nothing in the record to justify us in ignoring or declaring erroneous this specific finding of the District Court.

Defendant insists that the description of the patent is insufficient, under 35 U. S. C. A. 33, which requires inventors

to describe clearly their inventions. The District Court did not give any credence to this contention and we do not think we would be justified in declaring the patent invalid because of anything appearing in the record in this respect. True, routine experiments are to be employed, but these tests are conventional and well known to bacteriologists. The mere provision that the compound shall be made of strains or species of bacteria selected by familiar methods leaves nothing indefinite in the description. If noninhibitive strains were readily identifiable in nature as separate species, without scientific tests, of course tests would not be necessary, but no such good fortune confronts the scientist when he starts to isolate bacteria. There is no weight and measure method by which he may determine the quality or quantity of bacteria he is supposed to put into a composite product; there is available only selection by utilization of well known conventional tests. Only the scientist in his laboratory can produce controlled culture of bacteria and he, delving in the art of such culture, must at all times employ the methods found efficient by trained scientists. Where those methods are old and well known to the art, no difficulty is encountered in a description for a patent composite which provides for their utilization or employment. In other words, Bond's tests were manifest to readers skilled in the art. No one so skilled would have any difficulty in determining what he should do when his attention was directed to the necessity of making them. *Eibel Process Co.* v. *Minnesota & Ontario Paper Co.*, 261 U. S. 45, 63. As the Supreme Court has indicated, we think that intelligent bacteriologists, setting out properly to gather together the enumerated ingredients which are prescribed for the composite article, reading the description, could hardly go astray. *Seabury* v. *Am. Ende*, 152 U. S. 561. The requirement that preliminary tests must be made does not invalidate a specific method when their character and limitations are well known to the art. *Minerals Separation, Ltd.* v. *Hyde*, 242 U. S. 261; *A. B. Dick Co.* v. *Barnett*, 288 Fed. 799, 801 (CCA2).

Defendant contends that Bond merely taught how to make an aggregation lacking inventive character. We do not think so. An aggregation achieves nothing new. It is a term engrafted in the patent law either through the ingenuity of counsel or the lexicography of the courts, at a loss for a better term, to connote or express mere

lack of patentable inventive quality in a combination. As said by this court in *Lincoln Engineering Co.* v. *Stewart-Warner Corp.*, 91 F. 2d 757 (CCA7), the term "aggregation" if proper at all is proper as a definition of a phase of the term "non-invention." Where the claim constitutes a plurality of elements and their individual or collective selection fails to evidence the exercise of inventive faculty, it is of course not a patentable invention regardless of whether co-action of elements is present. On the other hand, an inventive concept may reside in the selection of a part or parts of numerous old elements. If the selection is unusual and a hitherto unsolved problem is overcome, it answers the tests of the statute and, in the absence of other limiting features, will achieve invention. So here Bond did a novel thing in the science of culture of bacteria to inoculate leguminous plants. He knew what other scientists already had suggested that certain strains worked adversely and detrimentally to each other. Delving further, he learned for the first time that various strains of the genus are non-inhibitive of other strains and can be grouped, with an entirely healthy result. As a result of his teachings, scientists learned how to make a composite inoculant without disastrous conflict of one species of bacteria with another. True, the composite inoculant had been thought of and practiced to some extent, but just as truly it had not achieved approved recognition. Indeed, scientists thought that it was a dangerous practice, and Bond, for the first time, pointed out to the bacteriologist the fact of existing noninhibitive strains and instructed him that he should select such strains and make his composite inoculant of them. Thus he taught how to make a composite inoculant, successful, efficient and filling the need it was meant to fill. This is not so-called "aggregation." It is inventive conception.

We now approach the issue of infringement. The District Court found "that the product • • • 'Legume-Aid' is a composite inoculant for leguminous plants consisting of a plurality of selected cultures of bacteria, comprising at least two species or strains of bacteria of the genus Rhizobium, which do not affect one another in their respective abilities to fix nitrogen in association with leguminous plants for which each species or strain is specific," and that "the accused composite culture • • •

'Legume-Aid' constitutes an infringement of each and all of said Claims 1, 3, 4, 5, 6, 7, 8, 13 and 14 of said Bond patent in suit." Defendant insists that there is no evidence to sustain these findings.

In *Gary Theatre Co.* v. *Columbia Pictures Corp., et al.*, 120 F. 2d 890, 894 (CCA7) and *Webb* v. *Frisch, et al.*, 111 F. 2d 887, 888 (CCA7), this court has previously discussed sufficiently the application of Rule 52 (a) of Rules of Civil Procedure to findings of fact such as the court entered here. If there is substantial evidence to support the conclusion, though we may not agree with the trial court, we are not privileged to disturb the findings unless they are clearly erroneous. And due regard is to be given to the trial court's opportunity to observe the witnesses and to judge their credibility. *Apex Electrical Mfg. Co.* v. *Maytag Co., et al.*, 122 F. 2d 182, 187 (CCA7) and cases there cited.

The burden rests upon a plaintiff to show that the defendant has made, used, or sold the patented invention; if the testimony is of equal weight the defendant must prevail. If the plaintiff makes out a prima facie case, however, the defendant must overcome it by evidence. Robinson on Patents, Vol. 3, Section 1041. Walker on Patents, Section 532. "Proof of the infringements given, that the machines made and used by the defendants were substantially like the complainant's, was sufficient, if not rebutted." *Bennett* v. *Fowler*, 8 Wall. 445; *Spring and others* v. *Domestic Sewing Machine Co.*, 9 F. 509; *Pfeifer* v. *Brown & Co.*, 85 F. 780. In a copyright case, *Encyclopedia Britannica Co.* v. *American Newspaper Ass'n.*, 130 F. 460, the court said: "Substantial identity, or a striking resemblance between the work complained of and that for which protection is claimed, creates a presumption of unlawful copying, which must be overcome by the defendant." To the same effect is *General Drafting Co., Inc.* v. *Andrews et al.*, 37 F. 2d 54 (CCA2).

When the burden of proof is once fixed as to a particular issue, in a strict sense, the burden of producing a preponderance of evidence never shifts. But there may be and often is a shift in burden of producing rebuttal evidence to escape an adverse decision based on an uncontested prima facie case, for, once such a case is made out, the burden of proceeding with further evidence rests on the opposite party. *United States* v. *Denver & R. G. R. Co.*, 191 U. S. 84,

92, 48 L. Ed. 106, 109, 24 Sup. Ct. 33; *Harrison* v. *New York Life Ins. Co.*, 78 F. 2d 421; *Toledo Metal Wheel Co.* v. *Foyer*, 223 F. 350; Cyclopedia of Federal Procedure, 2d Ed., Vol. 7, Sec. 3174. And it has always been the law that where the knowledge and means of proof with respect to an issue are peculiarly within the power and control of one of the parties, the burden of proof is, to that extent, placed upon him, although it would otherwise rest upon his adversary. Cyclopedia of Federal Procedure, Sec. 3174; *Miller* v. *Lykes Bros. Ripley S. S. Co.*, 98 F. 2d 185, cert. denied 305 U. S. 641; *United States* v. *Denver & R. G. R. Co.*, 191 U. S. 84.

In this case, by its response to plaintiff's request for admissions, Federal Rule of Procedure 36, defendant admitted that the accused product is a combination or mixed culture containing two species or varieties of root nodule bacteria, *i. e.*, Rhizobium trifolii (the clover bacteria) and Rhizobium meliloti (the alfalfa bacteria); that these two species of root nodule bacteria are contained in a moist powder base including humus; and that the accused Legume-Aid "is as efficient in its ability to fix nitrogen" in either clover or alfalfa plants as a single clover culture containing the same strains of clover bacteria is on clover plants and as a single alfalfa culture containing the same strains of alfalfa bacteria is on alfalfa plants. Defendant represents on its label that the two species of bacteria are "highly efficient selected strains." Defendant admits also that clover and alfalfa bacteria are present in the accused Legume-Aid in the proportions of 9 to 10 billion alfalfa bacteria and 14 billion clover bacteria per package, approximately the proportions recommended by Bond. Upon this showing plaintiff submitted its claim of infringement.

We have seen that Claim 1 defines a composite inoculant for leguminous plants comprising a plurality of selected cultures of different species of Rhizobium bacteria. Defendant's product, under its admission, contains the same. The patentee specifies that the cultures shall be "substantially unaffected by each other in respect to their ability to fix nitrogen in the leguminous plants for which they are specified" and the admission of defendant is that its composite of selected cultures of different species is as efficient to fix nitrogen in either clover or alfalfa as a single culture containing the same strains of clover bacteria is on clover plants and as single alfalfa species is on alfalfa plants.

Plaintiff's showing, therefore, made a prima facie case of infringement, and if the evidence stood alone, it would completely justify the finding of infringement made by the District Court. As the court commented in *Matheson* v. *Campbell*, 77 F. 280 at 284, plaintiff sustains the burden of proof, when it shows, as to infringement, that the alleged infringing product corresponds to the tests of identity. The burden is then upon the defendant to prove, if it can, that the product, though identical in composition, was not produced by the patented process. The further question presented then is as to whether defendant overcame this prima facie case, whether it has satisfactorily rebutted the presumption of infringement arising from plaintiff's evidence. That, obviously, is a question of fact, which, under the court's finding, has been decided adversely to defendant and which we are not justified in reversing unless we must say that, under the evidence presented, it is clearly erroneous.

We have examined the evidence and, though defendant insists that it does not make its composite product in accord with plaintiff's teaching and specifically denies that it makes any tests for the purpose of determining whether the different species are inhibitive or noninhibitive of each other, in view of all the evidence, we feel that we are not warranted in saying that the findings of fact are clearly erroneous. Defendant's assertion of noninfringement is based largely upon the testimony of Curie, an interested witness, in charge of manufacture of the accused product. He flatly denied that there are inhibitive bacterial strains. Other authorities disagree, and the trial court found his statement erroneous. Having denied that there are inhibitive strains, it followed, as Curie proceeded to say, that he never made any tests for inhibition or noninhibition. His testimony is not the most convincing. He admitted that he tested the mixed culture produced by defendant in an efficiency test as compared with a single culture. He admitted making tests with reference to legumes "to determine the efficiency of one strain of legume bacteria relative to others." He made "many nitrogen determinations of various legume plants." Whether he said he was testing for inhibition or testing for efficiency of composite product as compared with single products would seem to be of little importance. The question is, did he follow the teaching of Bond in determining what were efficient and nonefficient strains. He admitted that from time to time in the manufacturing

process; he discarded certain strains of bacteria as they proved to be no longer efficient or vigorous. He admitted making visual tests of the respective results of various cultures, contending that he never found undesirable or inefficient results exceeding from 10 to 20%, placing his mean at 15%. Obviously such a variation is of no small concern to the agriculturist who uses the product, if that variation is reflected in similar varying returns in crops. As a result of all this testimony, which we have read with care, we find ourselves still uncertain as to what Curie actually did and what the actual results of his tests were. At the most, we think we are not warranted in saying that the District Court clearly erred in concluding, after hearing Curie's testimony and observing him on the witness stand, that he had employed tests for efficiency of combined cultures in one composite product as compared with efficiency of single culture products and that these tests fulfilled the function of those prescribed by Bond. The evidence is not of such character that it is clear that the court was in error in its conclusion in this respect. We think the court was justified in finding that the patented process was substantially employed, especially when we remember that infringement is not negatived by mere avoidance of the best manner of practice or by failure to utilize the patent in its most advantageous form.

Remembering that identity of product raises a presumption of identity of process; that when a composition responds to the tests of identity prescribed by the patent, it is prima facie an infringement, as declared in *Matheson* v. *Campbell*, 77 F. 280; *Badische Anilin, etc. Fabrik* v. *Klipstein, etc., Co.*, 125 F. 543; *Holliday* v. *Schulze-Berge*, 78 F. 910, and that Curie's testimony in explanation is far from satisfactory rebuttal proof, we think that the District Court, who saw the witnesses and heard them testify, was not clearly in error in finding infringement.

The court dismissed the counterclaim of defendant for a declaratory judgment that all the claims of the Bond patent are invalid, being impelled to this action because it felt that, in view of its holding of invalidity of the claims in suit, there was no necessity of any adjudication upon the claims not relied upon by plaintiff. Claim 2, which was not presented in issue, is a product claim, similar to those in suit. Whether it is valid has not been adjudicated and is not before us. The other claims 9, 10, 11 and 12, are claims

for the method prescribed by Bond for the making of his composite inoculant. By the counterclaim defendant sought an adjudication that all claims are invalid or not infringed. Whether those claims are valid and whether defendant has infringed has never been decided because of the District Court's belief that it was unnecessary to make such decision. In a similar situation where the trial court held that, though by counterclaim defendant sought declaratory judgment, when the court found no infringement, there remained no justiciable controversy, the Supreme Court of the United States held in *Altvater* v. *Freeman*, 319 U. S. 359: "* * * To hold a patent valid if it is not infringed is to decide a hypothetical case. But the situation in the present case is quite different. We have here not only bill and answer but a counterclaim. Though the decision of noninfringement disposes of the bill and answer, it does not dispose of the counterclaim which raises the question of validity. *Sola Electric Co.* v. *Jefferson Electric Co.*, 317 U. S. 173, is authority for the proposition that the issue of validity may be raised by a counterclaim in an infringement suit. The requirements of case or controversy are of course no less strict under the Declaratory Judgments Act (38 Stat. 955, 28 U. S. C. Sec. 400) than in case of other suits. *United States* v. *West Virginia*, 295 U. S. 463, 475; *Ashwander* v. *Tennessee Valley Authority*, 297 U. S. 288, 325; *Aetna Life Ins. Co.* v. *Haworth*, 300 U. S. 227; *Maryland Casualty Co.* v. *Pacific Coal Co.*, 312 U. S. 270. But we are of the view that the issues raised by the present counterclaim were justiciable and that the controversy between the parties did not come to an end (*United States* v. *Alaska S. S. Co.*, 253 U. S. 113, 116) on the dismissal of the bill for noninfringement, since their dispute went beyond the single claim and the particular accused devices involved in that suit." *Schering Corporation* v. *Gilbert*, 153 F. 2d 428, 433; *Gebhard* v. *General Motors Sales Corporation*, 135 F. 2d 248, 249.

It has always been the desire of equity to dispose of all questions involved where it is at all possible to do so in order to prevent multiplicity of suits. So we think here that when plaintiff brought suit for infringement of certain claims and defendant counterclaimed seeking declaratory judgment upon all claims, the court, by judgment upon only the claims in suit, did not dispose of all issues in controversy between the parties. Defendant has a right to know

whether what it is doing violates any valid claim of plaintiff's patent. It has been confronted once with a suit for infringement and, though it does not manufacture, its principal, who defends, does manufacture and distribute, and, inasmuch as defendant itself distributes the inoculant, it has a right to know whether the product it does distribute infringes any valid claim. We think this is within the language of the Supreme Court as to what constitutes a justiciable controversy. The statute provides for declaratory judgment in the interest of prevention of multiplicity of litigation. We think the court below should have heard evidence upon and decided the counterclaim for a declaratory judgment.

The judgment is reversed in so far as it determined, (1), the claims in suit invalid, and, (2), dismissed the counterclaim, and is affirmed in so far as it disposed of all other issues.

And on the same day, to-wit: On the twelfth day of June, 1947, the following further proceedings were had and entered of record, to-wit:

Thursday, June 12, 1947.

Court met pursuant to adjournment.

Before:

Hon. Evan A. Evans, Circuit Judge.
Hon. J. Earl Major, Circuit Judge.
Hon. Walter C. Lindley, District Judge.

Kalo Inoculant Company, *Plaintiff-Appellant,*	Appeal from the District Court of the United States for the Southern District of Illinois, Southern Division.
9040 *vs.*	
Funk Brothers Seed Company, *Defendant-Appellee.*	

This cause came on to be heard on the transcript of the record from the District Court of the United States for the Southern District of Illinois, Southern Division, and was argued by counsel.

On consideration whereof, it is ordered and adjudged by this Court that the judgment of the said District Court in this cause appealed from be, and the same is hereby, reversed insofar as it determined, (1), the claims in suit invalid, and (2), dismissed the counterclaim. It is further ordered that the said Judgment be affirmed in so far as it disposed of all other issues.

It is further ordered that two-thirds (⅔) of the costs on these appeals be taxed against the Defendant, and one-third (⅓) against the Plaintiff.

Kalo Inoculant Company, *Plaintiff-Appellee,* vs. Funk Brothers Seed Company, *Defendant-Appellant.*	Appeal from the District Court of the United States for the Southern District of Illinois, Southern Division.
9041	

This cause came on to be heard on the transcript of the record from the District Court of the United States for the Southern District of Illinois, Southern Division, and was argued by counsel.

On consideration whereof, it is ordered and adjudged by this Court that the Judgment of the said District Court in this cause appealed from be, and the same is hereby, reversed insofar as it determined, (1), the claims in suit invalid, and (2), dismissed the counterclaim, It is further ordered that the said Judgment be affirmed in so far as it disposed of all other issues.

It is further ordered that two-thirds (⅔) of the costs on these appeals be taxed against the Defendant, and one-third (⅓) against the Plaintiff.

And afterwards, to-wit: On the twenty-seventh day of June, 1947, there was filed in the office of the Clerk of this Court a Motion for Stay of Mandate, which said Motion is in the words and figures following, to-wit:

IN THE UNITED STATES CIRCUIT COURT OF APPEALS

For the Seventh Circuit.

Kalo Inoculant Company, a corporation, *Plaintiff-Appellant, Cross-Appellee,* vs. Funk Brothers Seed Company, a corporation, *Defendant-Appellee, Cross-Appellant.*	Appeals Nos. 9040, 9041.

PETITION FOR STAY OF MANDATE.

Now comes the Defendant-Appellee, Funk Brothers Seed Company, and prays this Court for a stay of issuance of the Mandate in this cause (Appeals 9040 and 9041) for sixty (60) days pending application for a writ of certiorari to the Supreme Court of the United States.

Toulmin & Toulmin,
D. C. Staley,
Attorneys for Defendant-Appellee, Funk Brothers Seed Company.

B. A. Schroeder,
H. A. Toulmin, Jr.,
D. C. Staley,
Solicitors and Of Counsel for the Defendant.

Dayton, Ohio,
June 25, 1947.

A copy of this Petition is forwarded to Counsel for Plaintiff by First Class Mail.

Toulmin & Toulmin,
Attorneys for Defendant.

And on the same day, to-wit: On the twenty-seventh day of June, 1947, the following further proceedings were had and entered of record, to-wit:

Friday, June 27, 1947.

Court met pursuant to adjournment.

Before:

Hon. Evan A. Evans, Circuit Judge.

9040	Kalo Inoculant Company, *Plaintiff-Appellant*, *vs.* Funk Brothers Seed Company, *Defendant-Appellee.*	Appeals from the District Court of the United States for the Southern District of Illinois, Southern Division.
9041	Kalo Inoculant Company, *Plaintiff-Appellee*, *vs.* Funk Brothers Seed Company, *Defendant-Appellant.*	

On petition of counsel for Funk Brothers Seed Company, it is ordered that the issuance of the mandates of this Court in these appeals be, and the same is hereby, stayed for a period of sixty (60) days pending application to the Supreme Court of the United States for a writ of certiorari.

UNITED STATES CIRCUIT COURT OF APPEALS

For the Seventh Circuit.

I, Kenneth J. Carrick, Clerk of the United States Circuit Court of Appeals for the Seventh Circuit, do hereby certify that the foregoing printed pages contain a true copy of the papers filed and the proceedings had, concerning the hearing, the Opinion of the Court, Judgment, the Motion to Stay Mandate and the Order on the said Motion, as designated in the letter of counsel dated July 16, 1947, in:

Cause No. 9040-41.

Kalo Inoculant Company,
Plaintiff-Appellant,

vs.

Funk Brothers Seed Company,
Defendant-Appellee,

and

Kalo Inoculant Company,
Plaintiff-Appellee,

vs.

Funk Brothers Seed Company,
Defendant-Appellant,

as the same remains upon the files and records of the United States Circuit Court of Appeals for the Seventh Circuit.

In Testimony Whereof I hereunto subscribe my name and affix the seal of said United States Circuit Court of Appeals for the Seventh Circuit, at the City of Chicago, this 1st day of August, A. D. 1947.

(Seal)

Kenneth J. Carrick,
Clerk of the United States Circuit Court of Appeals for the Seventh Circuit.

SUPREME COURT OF THE UNITED STATES

ORDER ALLOWING CERTIORARI—Filed October 13, 1947.

The petition herein for a writ of certiorari to the United States Circuit Court of Appeals for the Seventh Circuit is granted. And it is further ordered that the duly certified copy of the transcript of the proceedings below which accompanied the petition shall be treated as though filed in response to such writ.

(3352)